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## RESEARCH HORIZONS

Five visions of the future

**US ELECTIONS AND SCIENCE**
To debate or not to debate

**REVOLUTIONARY THOUGHT**
Ten things to thank Darwin for

**HOLOGRAPHIC DISPLAYS**
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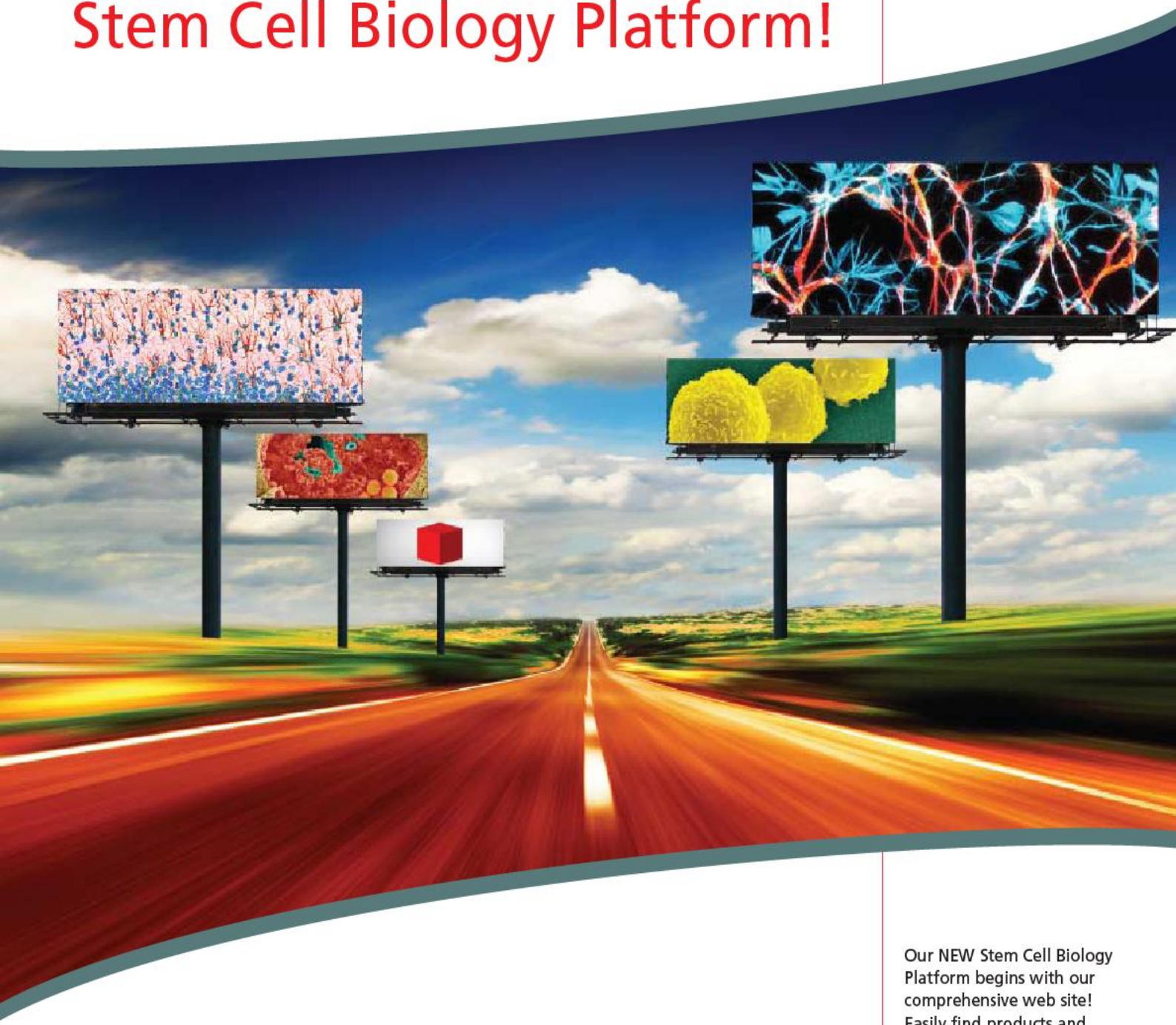
# Sigma-Aldrich Stem Cell Biology Platform!



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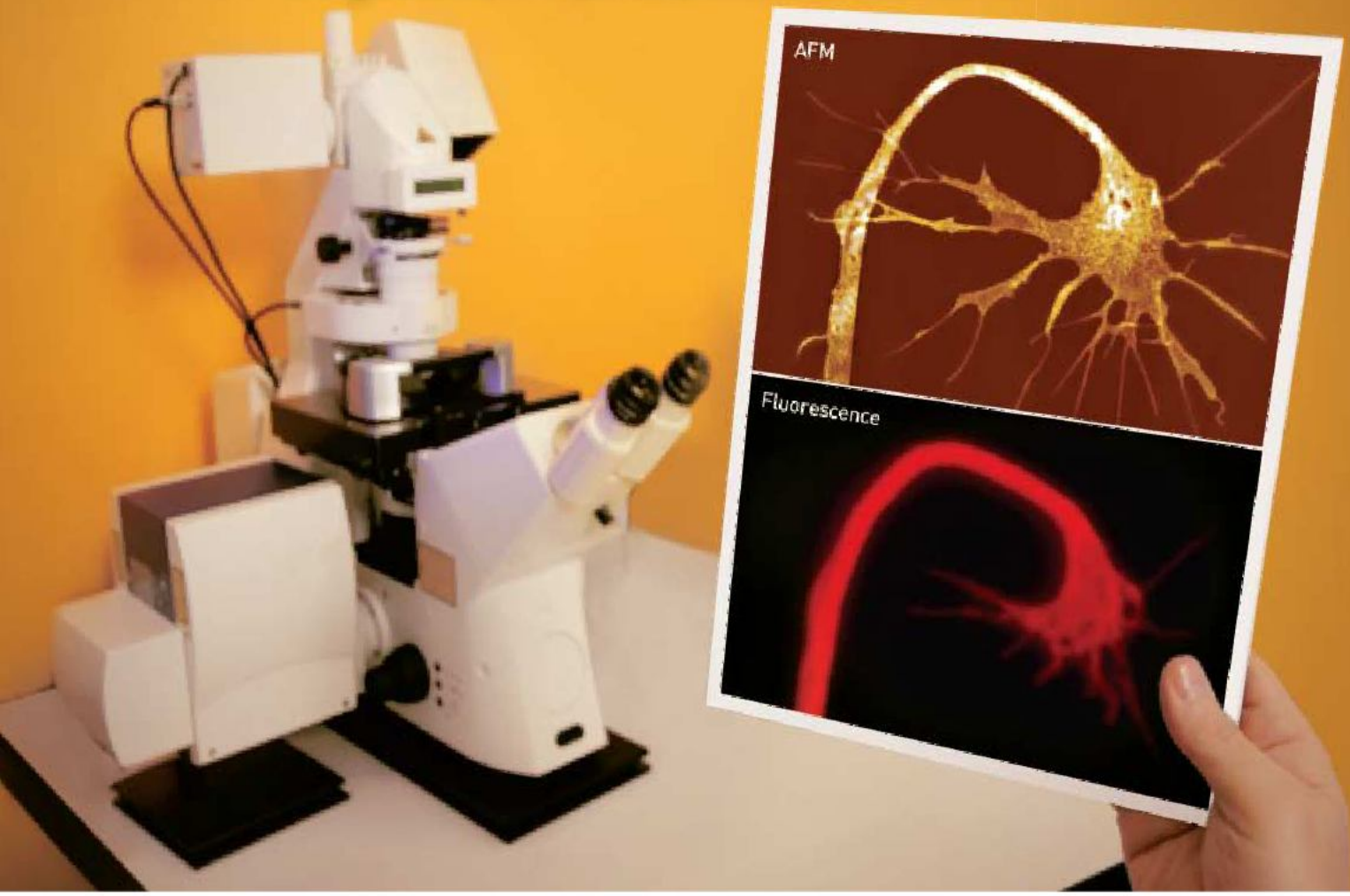


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## EDITORIALS



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## NEWS



## COLUMN



## NEWS FEATURES



## CORRESPONDENCE



## BOOKS & ARTS



## ESSAY




COLLECTIONSFP

The Belle Epoque in colour. Books & Arts p. **630**.

## NEWS & VIEWS



## HORIZONS



## NATUREJOBS



## FUTURES

# Nominations Open Now

## Manpei Suzuki International Prize for Diabetes Research

### 鈴木万平記念糖尿病国際賞

The Manpei Suzuki Diabetes Foundation, Tokyo, Japan is pleased to announce a new international prize for original and excellent achievements in basic and clinical diabetes research: The Manpei Suzuki International Prize for Diabetes Research.

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A Certificate of Honor and $150,000 USD prize will be awarded to one basic or clinical researcher working in the field of diabetes or related fields.

The prize will be presented in January, 2009.

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One flower bud opens to five petals.

— Bodhidharma

## Want to Nominate a researcher?

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**Nomination Deadline: May 31, 2008**

**Manpei Suzuki Diabetes Foundation**

12-5, 3-Chome, Akasaka, Minato-ku, Tokyo 107-0052 Japan

E-mail: secretariat@manpeizuzukiprize.org URL http://www.manpeisuzukiprize.org

R. RUNNING/SOLUS-VEER/CORBIS

Can systems biology make old age a healthier option? Horizons, p. **644**.

## REVIEW ARTICLE



## ARTICLES



## LETTERS



CCI ARCHIVES/SPL

False start — but battery power could yet rule the roads. Horizons, p. **652**.

# NATURE ONLINE

## ADVANCE ONLINE PUBLICATION

PUBLISHED ON 6 FEBRUARY 2008

**Formation and branch migration of Holliday junctions mediated by eukaryotic recombinases**
Y Murayama, Y Kurokawa, K Mayanagi & H Iwasaki
doi:10.1038/nature06609

**Haematopoietic stem cell release is regulated by circadian oscillations**
S Méndez-Ferrer, D Lucas, M Battista & P S Frenette
doi:10.1038/nature06685

## NEW HORIZONS

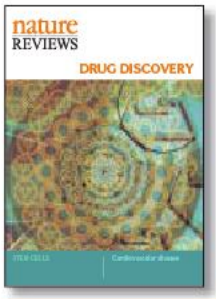
Five Horizons features this week kick off a new occasional *Nature* section, looking at the science that is to come. Our futurologists' attentions turn to ageing, 'evo-devo', battery power, 'open source' chemistry and the technologies needed for quantum computing. For more on these topics and the authors, download the latest podcast:
www.nature.com/podcast
And when you've listened to that, try Nature's Online Video Streaming Archive:
http://tinyurl.com/3dmj65

# This month in *Nature Reviews*


**FBW7 ubiquitin ligase: a tumour suppressor at the crossroads of cell division, growth and differentiation**
*M. Welcker & B.E Clurman*

How do the numerous cancer-associated mutations in FBW7 and its substrates contribute to tumorigenesis?

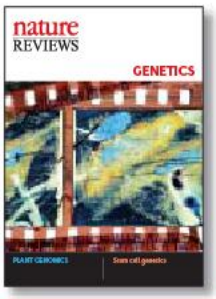
www.nature.com/reviews/cancer




**Derive and conquer: sourcing and differentiating stem cells for therapeutic applications**
*I. Klimanskaya, N. Rosenthal & R. Lanza*

This article discusses two key factors for the future use of stem cells in drug discovery and regenerative medicine: finding reliable sources of multipotent and pluripotent cells and the ability to control their differentiation to generate desired derivatives.

www.nature.com/reviews/drugdisc




**Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight?**
*W. Filipowicz, S.N. Bhattacharyya, & N. Sonenberg*

MicroRNAs regulate the expression of many genes in various organisms. This article, which is freely accessible in February, describes the progress made in defining the precise mechanism by which these molecules repress translation.

www.nature.com/reviews/genetics


**Shaping and reshaping CD8$^+$ T-cell memory**
*J. T. Harty & V. P. Badovinac*

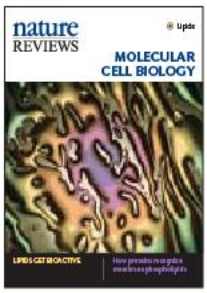
Here, John Harty and Vladimir Badovinac describe the factors that control the generation of memory CD8$^+$ T cells, discuss how these factors can characterize this response and highlight how the manipulation of these factors could reshape CD8$^+$ T-cell memory.

www.nature.com/reviews/immunology


**Exit strategies of intracellular pathogens**
*K. Hybiske & R. S. Stephens*

How intracellular bacteria exit host cells is a crucial stage in microbial pathogenesis that is driven by an evolutionary requirement for efficient dissemination to neighbouring cells and transmission to new hosts. In this Review, the authors discuss the strategies used by intracellular pathogens to escape their host cells.

www.nature.com/reviews/micro




**Lipid signalling in disease**
*M. P. Wymann & R. Schneiter*

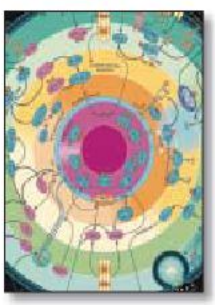
Lipids function as messengers in a lipid signalling network that controls many important cellular processes, including proliferation and apoptosis. Here, Wymann and Schneiter discuss how imbalances in this network contribute to the pathogenesis of different diseases, such as cancer and inflammation.

www.nature.com/reviews/molcellbio


**Interpreting fMRI data: maps, modules and dimensions**
*H. P. O. de Beeck, J. Haushofer & N. G. Kanwisher*

The ventral visual pathway contains both category-selective graded maps and distinct modules. The authors discuss the properties that define maps and modules, consider whether modules are parts of maps, and propose that different graded maps might combine to form discrete selective modules.

www.nature.com/reviews/neuro



***Nature Reviews Molecular Cell Biology* Focus on lipids**

Lipids, once thought only to separate extracellular and intracellular space, are now recognized as versatile molecules with diverse roles. The articles in this Focus consider different aspects of lipid biology, and a review of lipid signalling in disease is accompanied by a free Poster.

www.nature.com/nrm/focus/lipids
www.nature.com/nrm/posters/lipidsignalling-disease

# THIS ISSUE

**THE GREAT DEBATE DEBATE** Growing numbers of US science dignitaries are calling for a debate focusing on the remaining presidential candidates' views about science and technology. Our Washington diarist David Goldston, who was Chief of Staff of the US House Committee on Science from 2001 to 2006, begs to differ. [Party of One, p. **621**]

**ON THE CAMPAIGN TRAIL** A proposal by the New Jersey state legislature to issue bonds to pay for a $450-million investment in stem-cell research was blocked by voters at last year's elections following a successful campaign by conservative activists. Local scientists and politicians remain committed to the goal of making the state an important player in stem-cell research and plan to raise the issue again. In a News Feature, Meredith Wadman reports on their chances of reversing the verdict. [News Feature p. **622**]

**ORIGINAL THINKING** You'll be hearing a lot about Charles Darwin next year: 2009 is the bicentenary of his birth and the 150th anniversary of the publication of *Origin of Species*. Kevin Padian, for one, thinks that Darwin's contribution to Western thought in general, and science in particular, is well worth all the fuss. And to prove it, he offers a 'top ten' list of Darwin's achievements. [Essay p. **632**]

**LEARNING TO MANAGE** Fewer than 30% of PhD scientists go on to work in academia beyond a brief postdoc spell. Which is one reason why management training at graduate and postdoc level makes a lot of sense. And academic research labs need to be managed too. Genevive Bjorn reports on a growing trend. [Naturejobs p. **740**]

**BAD MEDICINE** In *Charlatan*, Pope Brock tells the cautionary tale of the rise and fall of John R. Brinkley, the notorious quack

KANSAS STATE HIST. SOC.

Caught red handed: Brinkley before his fall.

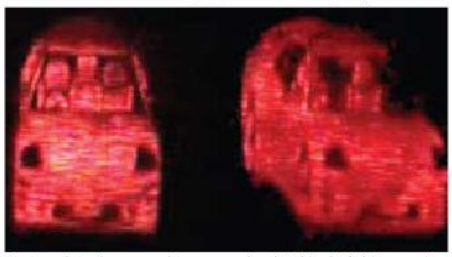
doctor operating in 1920s America who claimed that goat testicle implants were the fountain of youth. Couldn't happen today? Oh yes it could — every day on the Internet. [Books & Arts p. **628**]


ANDREW BARKER/ALAMY

A new series begins this week. 'Horizons' are commissioned articles in which experts speculate on what will happen over the next few years in their fields. On the cover, one of Antony Gormley's figures in his *Another Place* installation sets the tone. In the first piece, Thomas Kirkwood [page **644**] considers the potential of systems biology to de-link disease and old age. Peter Murray-Rust [page **648**] writes on a new 'open' approach to chemistry. But his subtext is broader: the future of the 'semantic web', where computers can make as much use of information as humans can. M. Armand and J.-M. Tarascon [page **652**] show how advances in materials science can provide the batteries of the future. George Koentges [page **658**] tackles 'evo-devo', the marriage of fossil evidence, genomic sequencing and molecular developmental biology. And R. J. Schoelkopf and S. M. Girvin [page **664**] raise the prospect that circuit quantum electrodynamics could pave the way for practical quantum computing and communication. On page **643**, *Nature* editor Philip Campbell sets out the brief for these and future Horizons.

## An added dimension

Three-dimensional holographic displays simulate natural human vision without the need for special eyewear. This makes them particularly suited to applications that require situational awareness, such as medical, industrial and military imaging. The current crop of commercial holographic 3D displays either



Just updated: new polymers make this kind of thing easier.

lack image-updating capability (so are 'write-once' devices) or have poor image persistence. Tay *et al.* now report the development of a recording medium based on specially designed photorefractive polymers that combines a number of favourable properties. They demonstrate a holographic 3D display based on this material that can record and display new images every few minutes, has a significant size (4×4 inch), can be viewed for several hours without the need for refreshing, and can be readily erased and updated with new images. [Letter p. **694**; News & Views p. **636**]

## Cracking zeolite catalysis

Zeolites are crystalline materials with ordered pore structures that are widely used as industrial catalysts. They are particularly important in oil refining, where the outcome of chemical transformations is strongly influenced by the pore topology of the zeolite catalyst. In a review, Berend Smit and Theo Maesen argue that this so-called shape selectivity can be rationalized using a straightforward thermodynamic analysis of how pore topology affects the free energies of formation of the reactants, intermediates and products. Despite the need for drastic simplifications, the approach can explain experimental observations and even guide the search for zeolite structures optimized for specific catalytic applications. [Review Article p. **671**]

## The ebb and flow of measles

Measles is all but eradicated in many countries, but in sub-Saharan Africa and parts of Asia it remains a major killer. An epidemiological study of measles in Niger between 1986 and 2002, just before a major immunization programme began, shows that the epidemics were unexpectedly episodic, interspersed with periods of local extinction. Modelling points to seasonality in disease transmission as the cause for this variability. This has implications for the management of vaccination campaigns. In particular, as vaccine delivery ramps up towards the goal of eradication, an occasional violent seasonal epidemic can be expected, and vaccination can be optimized to minimize this instability. [Article p. **679**]

## Two-paced plume

The Cassini mission discovered a spectacular plume of water vapour and icy dust particles spewing from ice volcanoes near the south pole of Saturn's moon Enceladus. The plume

has a puzzling property that has yet to be explained: the grains are moving more slowly than the vapour. Schmidt *et al*. present a quantitative model for the condensation of icy grains in the geysers of Enceladus that offers a possible explanation. The speed difference arises whilst the gas and dust are below the surface, where repeated wall collisions of grains combine with re-acceleration by the gas to cause friction that in turn reduces grain velocity. [Letter p. **685**]

## Time travelling proteins

Comparisons of genome sequence data in closely and distantly related modern organisms can be used for the computational reconstruction of ancient protein sequences that may have existed in related but now extinct types. These proteins can then be 'resurrected' in the laboratory. This has now been achieved for a group of 25 ancestral elongation factors from bacteria across an estimated span of 3 billion years. These ancient proteins display a near linear increase in thermostability travelling back in geological time, suggesting that the environment supporting ancient life was initially hot, then cooled progressively by about 30 °C during that period. This pattern is corroborated by the palaeotemperature trend inferred for the geologic record. [Letter p. **704**; News & Views p. **635**]

## Bacteria at sea

Metagenomics, or environmental genomics, has revolutionized our picture of microorganisms in the real world — as opposed to how they behave in laboratory cultivated 'clonal' cultures. A novel example of 'experimental metagenomics' is now reported, involving the creation of a 20-litre microcosm of sea water collected off Sapelo Island in the US state of Georgia. Manipulation of the system shows that this coastal microbial community is dominated by metabolic generalists capable of utilizing a wide variety of organic compounds, rather than by bacterial species that specialize in metabolizing a specific component of the dissolved organic carbon pool. This finding has important implications for identifying taxon–function relationships for carbon cycle-relevant processes and the construction of predictive models of ocean biogeochemistry. [Letter p. **708**]

## Recovery from N deposition

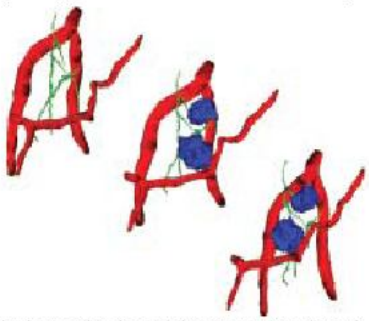
The use of nitrogen fertilizers and fossil fuel burning is causing nitrogen deposition in industrialized countries at up to seven times the pre-industrial rate. A long-term (23-year) study of prairie grasslands in Minnesota now suggests that the effects of such extended nitrogen deposition have been underestimated. Chronic low-level nitrogen addition led to a gradual loss of plant species diversity, but diversity had recovered 13 years after nitrogen addition had ceased, suggesting that some of the harmful effects of past deposition are reversed by reductions in the rate of deposition. [Letter p. **712**]

## Growing old fast

The rate at which the world's population ages is important to policymakers, especially those dealing with pensions and healthcare. In particular, it is vital to anticipate periods of rapid change, when adjustments will be the most difficult. A new estimate of ageing trends in the global population combines traditional measures, based on a fixed age boundary, with new concepts of age. New concepts include a fixed remaining life expectancy, as today's 60 year old is 'younger' than a 60 year old from 1900 and has more years left to live. No matter how you look at it, the estimates are that the world's population is ageing with increasing speed. Global ageing is likely to peak between 2020 and 2030, and then decelerate, although there will be further increases in the level of ageing throughout the century. The timing of 'peak ageing' is based on past patterns of fertility. In the United States and in parts of Western Europe the 'baby boomers' are a big factor. In China, the timing of fertility control policies has a big influence. [Letter p. **716**]

## Senile plaques: toxic combination

The senile plaques in brains of Alzheimer's disease sufferers are thought to develop gradually over years. The involvement of plaques in the pathology of Alzheimer's disease is a hotly debated issue. New work, using mutiphoton microscopy to follow plaque formation *in vivo* in a mouse model of the disease, supports the view that amyloid plaques appear before local neurotoxicity



Senile plaques (blue) and deforming neurites (green).

is visible. Surprisingly, senile plaques form rapidly, within 24 hours: within a day or two microglial cells accumulate and neuritic abnormalities start to appear. Based on these observations, it's possible to speculate that the slow degeneration in Alzheimer's disease is marked by sudden changes in cortical structure, and that altering the kinetics of this process might change the rate of disease progression. [Letter p. **720**; News & Views p. **638**]

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## Abstractions

**FIRST AUTHOR**

More than 40 years after the first measles vaccine became available, the virus is still a leading cause of death in children in many countries. Aid organizations such as Médecins Sans Frontières (MSF) are finding that vaccination strategies that proved successful in developed countries do not always work for developing regions. On page 679, Matthew Ferrari, a postdoc at Pennsylvania State University, and his colleagues show that seasonal and cultural patterns drive predictable episodic outbreaks of measles in Niger's capital, Niamey. In this setting, vaccination can be beneficial during outbreaks — a tactic previously considered inappropriate given the disease's rapid rate of transmission. Ferrari spoke to *Nature* about his work and its impact on health policy.

**Do organizations such as MSF typically reach out to academia?**

No. MSF focuses solely on providing health care, and has little opportunity to critically analyse data. They first contacted me to examine data from their vaccination programme in Niamey to assess whether supplemental vaccination in response to outbreaks might be beneficial.

**What challenges did you face when modelling measles outbreaks?**

Niger has one of the highest birth rates in the world, so there is a rapid build-up of children who are susceptible to measles. This makes it hard to determine the critical percentage of people that need to be vaccinated to head off an outbreak. MSF has a good relationship with the Niger Ministry of Health, and they granted us access to 20 years worth of measles incidence rates. Using these data, we showed that the classical dynamics of virus infection and spread do not hold up in Niger.

**What unexpected dynamics did you find?**

In Niger, the median age of measles infection is two years, whereas it is between five and six in European and North American countries, where children are socially protected until they enter school. We also found that the incidence of measles declines once the rainy season begins — presumably because the city's population falls when people return to agriculture after the dry season. We are now looking at satellite imagery to better understand how changes in population density affect disease transmission.

**Has this work changed your career goals?**

Yes. I now want to work at institutions with strong public health connections. I like the rapid interplay between data gathering and critical evaluation of health policy, because I think it keeps scientists accountable — a strong motivator to do good work. ■

## MAKING THE PAPER

Christopher Clark

**Soil nitrogen's detrimental effects on plant diversity may be reversible.**

Few scientists can say that their research project grew up as they did. But when ecologist Christopher Clark joined David Tilman at the University of Minnesota, St Paul, as a graduate student in 2001, Tilman's prairie grassland project to understand the effects of nitrogen deposition had already been running for almost 20 years. "I was 7 years old when Dr Tilman started it," says Clark.

Nitrogen is an essential element for plants, but too much of it in the soil results in decreased plant biodiversity. Nitrogen also affects the global carbon cycle. Normally, soil holds twice as much carbon as the atmosphere. The presence of extra nitrogen may cause less carbon to be stored in soil, contributing to global warming.

During the past 50 years, fossil-fuel combustion and fertilizer use have greatly increased the amount of nitrogen deposition in land ecosystems. "It has been estimated that human activities rival all natural processes combined in terms of how much nitrogen is being put into ecosystems," says Clark. "We're basically doubling the nitrogen supply globally."

Tilman's project to study the effects that nitrogen is having on plant biodiversity began in 1982. He deposited varying amounts of nitrogen-containing fertilizer onto a patchwork of grassland plots in three fields of Cedar Creek Natural History Area in Minnesota every summer until 2004. The fields were chosen because they had fallen out of agricultural use, and had become dominated by a species-rich mixture of native grasses and forbs. During the spring and summer months, Tilman — and later Clark — and various teams of summer interns harvested and recorded the plants that had emerged in the various plots.

In their analysis, described on page 712, Clark and Tilman found that even the lowest amounts of nitrogen in their treatments, which mimicked rates of nitrogen deposition over much of the developed world, resulted in the loss of 1 in 6 plant species. This is a large attrition, says Clark, because there are hundreds of plant species at their sites. Contrary to expectation, there was little difference in the loss of biodiversity between sites that received high and low nitrogen input. Clark suggests that because most species in this region are adapted to grow in nutrient-poor conditions, nitrogen inputs at any rate above the low historical levels may have an effect on the plant community.

But the study's results do not bear only bad news. From 1992, as part of a second experiment, Tilman stopped treating half of the plots in one of the three fields with fertilizer. Thirteen years later, he and Clark found that changes in biodiversity in these plots occurred at the same rate as in plots that had never received any fertilizer. "It may take a while, but our ecosystem seems to be able to recover," says Clark, now a newly minted postdoctoral fellow in Jianguo Wu's lab at Arizona State University in Tempe. He is about to embark on a biodiversity study of the Eurasian Steppe in China.

"Our findings suggest that if we start some sort of coordinated national–international effort we can either prevent or reverse some of these losses, which are probably occurring across much of the globe," Clark says. ■

## FROM THE BLOGOSPHERE

A Commentary suggesting widespread duplicate publication (*Nature* **451,** 397–399; 2008) has caused a storm of responses. Reactions across the NPG blogs and forums are captured on Nautilus (http://tinyurl.com/39b7gt).

The Publishing in the New Millennium forum on Nature Network reports an informed and passionate debate among scientists about whether duplicate publication is a problem in their fields and, if it is, how it can be stemmed. And at the Nature Precedings forum, Hilary Spencer asks whether posting papers on a preprint server — previously suggested to serve as a possible check and balance in the peer-review system — may encourage plagiarism. Publishers can search for duplicates among manuscripts submitted to their own journals, but a plagiarism-detection system across all publishers (http://tinyurl.com/3y9tan), currently in trial, might be more useful. It will, however, add to publication costs.

For authors wishing to submit to Nature journals, the editors provide guidance on issues including plagiarism and due credit for unpublished data at our Authors & Referees' website (http://tinyurl.com/3bmo5a). ■

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## EDITORIAL

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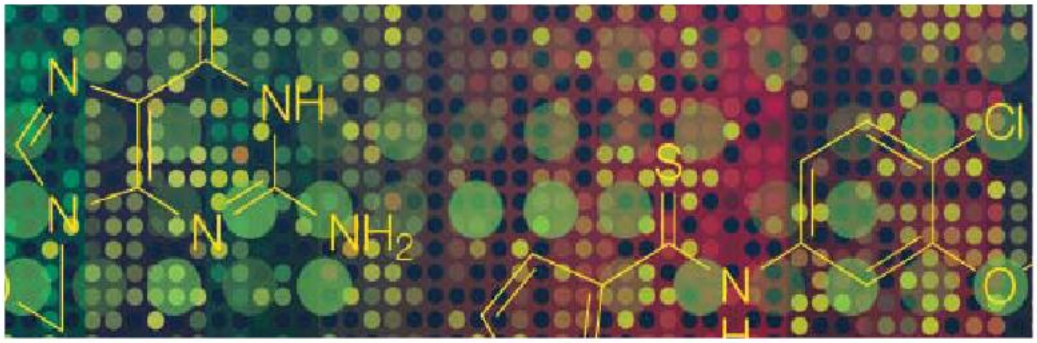

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Vol 451 | Issue no. 7179 | 7 February 2008

# Best tests for candidates

Science in presidential debates? Absolutely. A science debate? Not so sure.

Many of the great and good in US science — from the National Academies to Nobel laureates and various journals, including some parts of the Nature Publishing Group — have joined an initiative calling for the American election campaigns to feature a science debate. Such is the groundswell of support that their call is starting to feel like an idea whose time has come, and indeed it may prove to be so. You can join the throng at www.sciencedebate2008.com.

But in true scientific spirit, the proposal itself requires critical scrutiny — see, for example, page 621. And the campaign's website goes too far in saying that science and technology "may be the most important social issue of our time". In reality, science and technology are a factor in many issues, sometimes a defining one, but most often not. They can and must inform political debate, but will rarely be at its centre.

Take the key issue of climate change, which is at the top of the science debate list. The Bush administration's self-interested denialism and subsequent heel-dragging have infuriated informed opinion at home and abroad. But this anger, widely felt by scientists and others, should not lead us to raise science above other concerns out of a sense of slight. The science of the Earth system is crucial to understanding climate change; that does not mean that climate is best debated as a science issue.

Climate change should indeed be debated by the ultimate contenders for the presidency. The optimum format would allow them to question each other freely, with expert interlocutors able to challenge claims and highlight both common ground and inconsistencies. Scientific issues — how to deal with the uncertainties of climate sensitivity when deciding goals for emissions, or how far to shift federal research priorities towards near-to-medium-term innovation in alternative-energy systems — would play a key role in such a debate. But they would not be the whole story: tax policy, international trade, treaty law and foreign policy are just as crucial.

A similar approach, with candidates interacting with experts as well as each other, could be applied in other areas that are both of concern to scientists and significantly dependent on scientific data and research. The provision of health care, the encouragement of economic growth and the avoidance of nuclear proliferation are obvious possibilities.

Tests of the candidates' mettle might go further. In 2001, Democratic senator Sam Nunn played the role of the president in a much reported war-game, Dark Winter, which simulated a bioterrorist smallpox attack (the terrorists won). If the voters want a sense of how the presidential hopefuls respond to rapid influxes of technical expertise, why not ask the candidates and their chosen staff to submit separately to some similarly gnarly scenario containing a strong dose of science in front of the cameras. Aired back-to-back, the war games would undoubtedly make riveting viewing, and might reveal more about a candidate's relation to scientific expertise — knowing how to ask questions, how to value responses, how to face uncertainties — than an opportunity to discuss the science budget (see page 610).

**"Science and technology can and must inform political debate, but will rarely be at its centre."**

Turning a presidential campaign into a reality-TV version of *24* is obviously far fetched, and not necessarily desirable. But any sort of science debate is quite a stretch from business-as-usual. Well meant though it may be, the idea of Tim Russert or some other journalist-interrogator looking Republican hopeful John McCain in the eye and asking "What balance will you seek in federal science funding between major-programme project research and investigator-initiated basic-research grants?" is somewhat fantastical.

It is also slightly disturbing. For all that it claims to be a 'grass-roots' phenomenon, the proposed debate can be seen as an attempt by various élite institutions to grab the microphone and set the agenda from the top down.

Proponents might respond that this is the only practical way in which these issues can be raised. If so, any success of the debate proposal will mark a failure as well: a long-term failure to have the important contribution of science play an appropriate role in all levels of political discourse, which cannot be blamed solely on the Bush administration. To rectify that will require the long-term and possibly grubby work of cultivating broad political constituencies. But that offers more certain sustainability. The debate, if it happens, may be a grand thing on the night, but what will it change? ■

# Working double-blind

Should there be author anonymity in peer review?

Double-blind peer review, in which both authors and referees are anonymous, is apparently much revered, if not much practised. The Publishing Research Consortium (PRC) has assessed attitudes towards peer review among 3,000 academics in an international survey across the sciences and humanities. The results, released last month[1], strongly affirm the value of peer review. They also highlight that 71% have confidence in double-blind peer review and that 56% prefer it to other forms of review. Support is highest with those who have experienced it (the humanities and social sciences) or where it is perceived to do the most good (among female authors). The least enthusiastic group is editors. So is it time for editors, and those at *Nature* in particular, to reconsider their position?

If referees know the authors' identities, it may leave the latter vulnerable to biases about them or their previous work, their gender, their nationality or their being new to an area of research. But the PRC survey supports the contention of *Nature* and others that identifying authors stimulates referees to ask appropriate questions

(for example, differentiating between a muddy technical explanation and poor experimental technique). Knowing author identities also makes it easier to compare the new manuscript with the authors' previously published work, to ensure that a true advance is being reported. And knowing rather than guessing the identities of authors encourages reviewers to raise potential conflicts of interest to the editors.

Is there evidence that double-blind peer review presents a better alternative? It would do so if it generated more constructive comments in the minds of editors and authors, or if the identity of authors were truly protected, or if biases were reduced. So far, the jury is out. Although at least one study in the biomedical literature has suggested that double-blind peer review increases the quality of reviews, a larger study of seven medical journals[2,3] indicated that neither authors nor editors found significant difference in the quality of comments when both referees and authors were blinded. Referees could identify at least one of the authors on about 40% of the papers, undermining the *raison d'être* for double-blinding. The editors at the Public Library of Science abandoned double-blind peer review because too few requested it and authors were too readily identified.

The one bright light in favour of double-blind peer review is the measured reduction in bias against authors with female first names (shown in numerous studies, such as ref. 4). This suggests that authors submitting papers to traditionally minded journals should include the given names of authors only on the final, published version.

The double-blind approach is predicated on a culture in which manuscripts-in-progress are kept secret. This is true for the most part in the life sciences. But some physical sciences, such as high-energy physics, share preprints extensively through arXiv, an online repository. Thus, double-blind peer review is at odds with another 'force for good' in the academic world: the open sharing of information. The PRC survey found that highly competitive fields (such as neuroscience) or those with larger commercial or applied interests (such as materials science and chemical engineering) were the most enthusiastic about double-blinding, whereas fields with more of a tradition for openness (astronomy and mathematics) were decidedly less supportive.

Where does this leave journals? Editors have the responsibility to provide a neutral bridge between referees and authors and so may help to better shield authors from bias. Easily said! The evidence of the PRC survey suggests little faith in that impartiality, but editors — certainly at *Nature* and its related journals — take that responsibility seriously.

*Nature*'s policies over the years have generally moved towards greater transparency. Coupling that with the lack of evidence that double-anonymity is beneficial makes this journal resistant to adopting it as the default refereeing policy any time soon. But many of our readers are referees as well as authors. We welcome their views on author anonymity from both vantage points. To that end, this Editorial will be posted for comment at http://blogs.nature.com/peer-to-peer/2008/02/working_doubleblind.html. ■

1. Publishing Research Consortium *Peer Review in Scholarly Journals* (Mark Ware Consulting, Bristol, 2008); available at http://www.publishingresearch.net/PeerReview.htm
2. Justice, A. C. *et al. J. Am. Med. Assoc.* **280,** 240–242 (1998).
3. Cho, M. K. *et al. J. Am. Med. Assoc.* **280,** 243–245 (1998).
4. Budden, A. E. *et al. Trends Ecol. Evol.* **23,** 4–6 (2008).

# Don't ban labels

## Providing context for sensitive declarations is the job of industry and government.

'You are what you eat' notwithstanding, it is only recently that most consumers have become interested in the technical details of their food's composition, production and transport. With obesity and climate change now major concerns, and 'localvore' and 'food miles' entering the lexicon, shoppers are clamouring for information. And many food companies are happy to supply it, resulting in a dizzying array of multicoloured labels and claims.

But not everyone is happy. A proposed law in Indiana is the latest attempt in the United States to ban milk labels proclaiming that the cows from whence the milk came were not treated with recombinant bovine growth hormone (rBGH, also called recombinant bovine somatotropin or rbST). This hormone is produced by engineered bacteria, is virtually identical to the cow's own and can increase milk production by 10–15%.

There are two bad arguments for banning such labels. The first — that it is impossible to determine from the milk whether the cow was injected with rBGH — is the reason cited in the bill language. The second — that a proliferation of 'no rBGH' labels will train consumers to distrust the product — is the real motivation.

The first argument can be disposed of easily: it is already illegal to make false claims about a product. The second argument may seem more convincing. There is no firm scientific evidence that injecting cows with rBGH affects human health in any way, but prevalent labelling touting the absence of rBGH would suggest to consumers that there are some differences. The mandating of an additional phrase such as that agreed last month in Pennsylvania — "No significant difference has been shown between milk derived from rbST-treated and non-rbST-treated cows" — ameliorates this problem.

The hormone injections may not affect the milk, but they are rough on the cows: producing all that milk causes problems such as udder infections and lameness. For some consumers, this may be a sufficient reason to avoid milk from dairies using the injections. Indeed, it was, in part, animal welfare that led Canada and the European Union to ban them.

There are good reasons not to ban accurate labels. More information means that consumers can be more discerning, and not just about their own health. They can vote with their purchases for farming practices they prefer. And if a company wants to use a technology with a bad reputation, it is the firm's responsibility to educate the consumer about why it is beneficial. If consumers choose irrationally to reject it, that is their prerogative. Capitalism thrives on the irrationality of consumers, from their noted fear of smelling bad, to their preference for redness in apples, farmed salmon and fast-food signage.

Indeed, if consumers were suddenly to become rational, an economic cataclysm would result, as households in all the rich nations would cut their consumption to only what they really needed. Such a crash would no doubt make the current economic doldrums look like the mildest hiccup. ■


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# RESEARCH HIGHLIGHTS

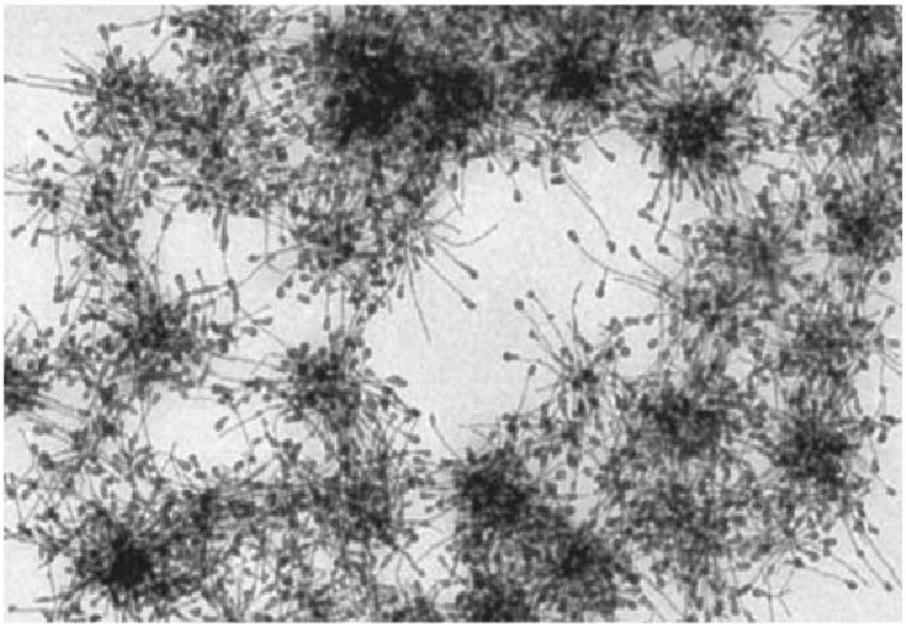
## Gold webs

*Adv. Mater.* doi:10.1002/adma.200701518 (2008)

A duo in Germany has glued gold particles to an unusual nanoscale crystal cobweb, creating a structure that could lead to more efficient solar cells.

Yuriy Khalavka and Carsten Sönnichsen at the University of Mainz grew cadmium telluride crystals that branch into cobweb-like tangles of crystalline filaments. The material is a semiconductor, and organic compounds, such as those used in photovoltaics, can get inside the tangles. Thus, if these inorganic crystals were hooked up to other electronics, they could be used as scaffolding in new types of solar cell.

The researchers have created electrical contacts on the cadmium telluride tangles by bonding gold atoms to the crystals' tips (pictured). They now plan to measure the tangles' electrical properties using these gold leads.



Y. KHALAVKA

CHEMICAL BIOLOGY

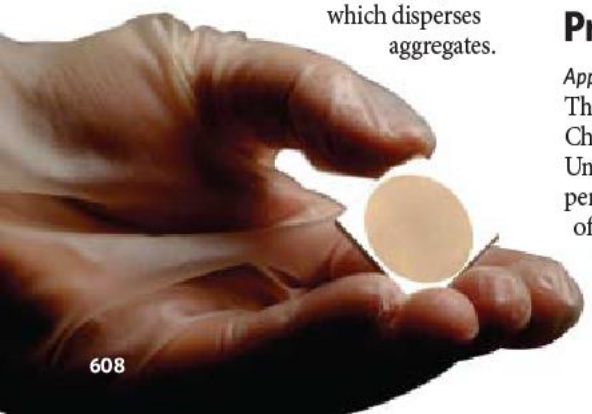
## Aggravating aggregating

*Nature Chem. Biol.* doi:10.1038/nchembio.65 (2008)

Several early drug candidates that prevent the formation of protein fibre aggregates known as amyloids in the brain may act nonspecifically, making them unsuitable for treating disorders associated with amyloid accumulation such as Alzheimer's disease and prion diseases, researchers caution.

Many amyloid inhibitors are structurally similar to chemical aggregators, compounds that clump together in solution. Brian Shoichet of the University of California, San Francisco, and his colleagues proposed that amyloid inhibitors could function nonspecifically by sequestering individual proteins in such clumps, and therefore keeping them out of amyloids.

The researchers found that eight chemical aggregators inhibited polymerization of a yeast prion protein. Several of these compounds also prevented prion infection in live yeast cells. In addition, five known amyloid inhibitors were not able to function when they were prevented from clumping by the introduction of detergent, which disperses aggregates.



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PALAEOBIOLOGY

## Deep-sea damage

*Proc. Natl. Acad. Sci. USA* doi:10.1073/pnas.0705486105 (2008)

Populations of small crustaceans called ostracodes living deep in the ocean collapsed after major climatic events during the past 20,000 years, according to Moriaki Yasuhara of the US Geological Survey in Reston, Virginia, and his colleagues.

Climate and deep-ocean circulation are known to be linked, but whether climate change could seriously affect deep-sea ecosystems has been a matter of debate. The authors measured the diversity of ostracode fossils in a deep-sea sediment core from the northwestern Atlantic Ocean. They found that ostracode species richness has regularly plunged to as little as half its normal level following abrupt alterations in Earth's climate.

The findings do not prove that deep-sea ecosystems are destined for damage in the face of anthropogenic global warming, but they posit the likelihood of it happening.

METALLURGY

## Pretty on the outside

*Appl. Phys. Lett.* doi:10.1063/1.2834902 (2008)

The alchemists would be green with envy. Chunlei Guo and Anatoliy Vorobyev of the University of Rochester in New York can permanently change the surface colour of metals such as aluminium, which is normally silver-coloured, to gold (pictured, left) — and to grey and black. Their trick is to etch the metals' surfaces with pits of different lengths and create other tiny shapes using a powerful laser. These tune the surfaces to absorb particular wavelengths of light, and reflect only the desired colour — or almost no light in the case of black.

At the moment, the process takes four hours to cover a few square centimetres of metal. But with some improvements to speed up the method, blackened metals could prove useful parts of stealth aircraft, colourful metals may replace filters in telescopes and, for the romantic goth, gold wedding bands could be turned black, says Guo.

GENOMICS

## Inner differences

*Nature Genet.* doi:10.10.1038/ng.78 (2008)

A group at the Pasteur Institute in Paris has identified regions of the human genome that probably contribute to differences in appearance, disease susceptibility and other physical traits among four human populations.

Pygmies are small and Massai are tall, northern European adults are good at digesting milk and west Africans have a decent chance of resisting malaria. But the genetic data allowing us to pick apart where natural selection has chipped away at the genome and created such differences only became available with the publication of the HapMap.

Lluís Quintana-Murci and his colleagues analysed more than 2.8 million single-base changes in the genomes of Han Chinese, Japanese, northwestern Europeans and Nigerian Yoruba. The genetic differences

associated with each of these ethnic groups should suggest candidate genes for medical conditions that burden some populations more than others, the authors conclude.

LINGUISTICS

## Lingua frantic

*Science* 319, 588 (2008)

If all language evolved at the same stately pace, then the number of words that differ between any two languages would be easily calculated by multiplying this constant by how long ago the two tongues parted ways. But Mark Pagel at the University of Reading, UK, and his colleagues have found that branches heavy with linguistic divorces evolve faster, suggesting that 'punctuational bursts' of language change occur just after splits happen. The authors calculate that the rapid change during these bursts accounts for 10–33% of the differences between languages.

Pagel and his team suggest two possible reasons for such bursts: founder events in which the idiosyncrasies of a small number of language 'originators' permanently colour the language, or the desire of recently separated groups to establish distinct identities.

ZOOLOGY

## High pitch

*Proc. R. Soc. B* doi:10.1098/rspb.2007.1619 (2008)

The male Anna's hummingbird (*Calypte anna*, pictured right) has an impressive trick that seems to be for wooing the opposite sex: it swoops down in a graceful dive accompanied by a loud chirp as high as the top note on a piano. Oddly, this sound is produced not vocally, but by the bird's tail feathers, Christopher James Clark and Teresa Feo report.

The mechanism, say the researchers from the Museum of Vertebrate Zoology at the University of California, Berkeley, is similar to a flag making a flapping sound in the wind. But according to their high-speed video analysis, the speed of the dive is so rapid, and the flapping frequency of the bird's tail feathers so high and so finely tuned, as to make a single clear note ring out.

CELL BIOLOGY

## Another gift from Mum

*Science* 319, 613–616 (2008)

Pig and mouse embryos require nucleoli provided by the egg to survive early development, researchers in Europe and Japan have discovered. Nucleoli are spherical organelles that make parts for cellular protein factories called ribosomes, and are found inside the nuclei of most organisms other than bacteria and archaea, which lack a true nucleus. They vanish during sperm maturation, but whether the sperm's genetic information might allow their synthesis later in the embryo was unknown.

Sugako Ogushi at Kobe University in Japan and her collaborators removed nucleoli from unfertilized oocytes using microsurgery. All the embryos formed from these enucleolated eggs stopped developing after only a few cell divisions.

Proper development could be restored by reinjecting nucleoli from other eggs but not by transferring nuclei from either somatic or embryonic stem cells, showing that successful cloning requires intact nucleoli from eggs.

C. CLARK/A. VARMA

BIOCHEMISTRY

## Paired pairs

*J. Phys. Chem. B* 112, 1060–1064 (2008)

Two strips of double-stranded DNA can stick together in a manner that depends on the sequences of their bases, Geoff Baldwin of Imperial College London and his colleagues have found.

That a single DNA strand can stick to a double helix by hydrogen bonding was already known, but the idea that two double helices pair up according to their sequences is new. Baldwin and his co-workers fluorescently tagged two DNA duplexes of the same length and nucleotide composition, but with different sequences, and found that the two types of molecule paired up, like with like, in liquid-crystalline aggregates when mixed together in an electrolyte.

Subtle, sequence-dependent differences in the space between the 'screw threads' of the duplexes may affect how the coils fit together, and thus the electrostatic interactions between them. The effect may explain some mysterious features of DNA recombination in cells.

## JOURNAL CLUB

**Gerald Crabtree**
**Howard Hughes Medical Institute, Stanford University School of Medicine, California**

**A developmental biologist muses on the magic of the egg.**

Many biologists, myself included, grew up watching frogs' eggs hatch into tadpoles at the warm surfaces of summer ponds. The yearly cycle provided a leisurely period of thought about basic biology. But few of us guessed how central to current biological and financial interests the egg would become. These days, an enucleated egg's ability to reprogramme the nucleus of a somatic cell — first demonstrated in frogs' eggs in 1958 — promises an era in which organs could be picked up like junkyard parts.

What magic does the egg possess that allows it to reset the nucleus to a basal, or 'pluripotent', state from which all cells can be generated? The three famous transcription factors — Oct4, Sox2 and Klf4 — that are required to transform a skin cell into a pluripotent cell provide some insight. But do these recapitulate a pattern used by the egg during development, or induce reprogramming by an alternative pathway?

John Gurdon and his colleagues at the Gurdon Institute in Cambridge, UK, have purified the proteins that bind to the regulatory sequences of the *Oct4* gene in frogs' eggs (M. J. Koziol *et al. Curr. Biol.* 17, 801–807; 2007). The group chose *Oct4* because its regulatory regions have been clearly defined. They found that the initiation of *Oct4* expression involved, in addition to likely candidates, some unexpected proteins.

If, as many scientists think is the case, the re-establishment of pluripotency involves short-circuiting egg development, this suggests to me that the magic that allows the egg to reset a nucleus into a pluripotent state may lie in these unexpected proteins — as well as Oct4, Sox2 and Klf4. There is so much more to learn from watching frogs' eggs grow up.

Discuss this paper at **http://blogs.nature.com/nature/journalclub**

NEWS

# Bush asks for more physics — again

**WASHINGTON DC**

In his final year as president, George W. Bush has put forward a budget wish-list that looks to restore his priorities in science and research, with solid increases for some physical sciences and pretty much no new money for the biomedical sector. Whether Congress will go along with this remains to be seen.

In terms of research and development, the budget's most pronounced feature is a 15% (US$1.6 billion) increase in physical-sciences spending year on year (see table, opposite). In December 2007, last-minute negotiations in Congress derailed the second year of Bush's 'American Competitiveness Initiative', removing money from many physical-science programmes. This new request is meant to put the initiative's aim of a doubling of physical-sciences budgets over 10 years as part of the initiative back on track, asking for $12.2 billion spread across the National Science Foundation (NSF), the Department of Energy's (DOE's) Office of Science, and the National Institute of Standards and Technology (NIST). At the same time Bush would freeze funding for the National Institutes of Health (NIH) at $29.3 billion.

So the NIH budget, which doubled between 1998 and 2003, is being allowed to stagnate, while the administration tries to put the physical sciences on their own doubling path. According to Robert Berdahl, president of the Association of American Universities, which works to increase science funding across the board: "There's the sense that 'We took care of the NIH, now we're going to take care of the physical sciences'. The sad part of that is we're losing the effect of the doubling on the NIH."

"I think it was a mistake to double the NIH budget in just five years without a plan to change how that money was spent," says John Marburger, the president's science adviser, referring to the ramp-up begun under President Clinton in 1998. "We're now seeing the consequences." He says that the ramp-up in the smaller physical-sciences budget will be slower, and thus offer more sustainable gains.

It remains to be seen whether Congress will meet Bush's wishes for the physical sciences; last year it didn't, instead slashing the proposed competitiveness increases. The president's annual budget request sets off a months-long appropriations process in which Congress sets the final numbers; those generally track relatively close to what the president asked for (see 'Granting requests'). But in an election year like this, Berdahl notes, partisanship increases. Bart Gordon, a Tennessee Democrat who chairs the

J. WATSON/AFP/GETTY

President Bush outlined his priorities at the State of the Union address — but will Congress agree?

E. MAYNARD/ALAMY

**MEETING REPORT FROM THE AAAS**
Visit our events blog from 14 February for reports
http://blogs.nature.com/news/blog

House science committee, called the request "an incomplete and short-sighted plan" that did not include enough money to spur education and innovation in industry. So it is entirely possible that congressional Democrats, waiting to see who becomes president in November, could force another end-of-year budget showdown.

At the DOE, the president's budget would boost spending for 'clean-coal' technologies from $520 million to $648 million, even though the department is pulling the plug on FutureGen, a flagship carbon-capture and -storage project (see page 612). Nuclear energy would increase from $755 million to $932 million including $302 million for reprocessing technologies related to the Global Nuclear Energy Partnership, over which Bush and Congress have battled.

After a year of hardship, fusion and high-energy physics would see significant gains within the DOE's Office of Science. The appropriations bill passed in December held almost no money for ITER, the international fusion research reactor in France, and left physicists at the Fermi National Accelerator Laboratory in Batavia, Illinois, facing lay-offs and mandatory unpaid leave. The new budget holds $214 million for ITER and would allow Fermilab to resume work on the NOvA neutrino research programme. There would also be $35 million for research for the proposed International Linear Collider, another victim of the December settlement. "It's a good budget for particle physics," says Fermilab director Pier Oddone.

Over at the NSF, officials were also upbeat. With $6.85 billion budgeted, the agency would receive a 13.6% boost over the 2008 levels approved by Congress. "I am optimistic that the NSF will regain its budget momentum in 2009," says Arden Bement, the agency's director. In line with the emphasis on physical-sciences research, three directorates — computer sciences, physics and engineering — would see their budgets increase by nearly 20%. Large facilities such as the ALMA telescope array would continue on track. And overall the agency expects to issue 1,370 more research grants if the request is approved, boosting the application success rate slightly from 21% to 23%.

US SCIENCE AND TECHNOLOGY BUDGET

| Agency | FY08 request (in millions of dollars) | FY08 enacted | FY09 request |
|---|---|---|---|
| National Institutes of Health | 28,699 | 29,307 | 29,307 |
| NASA | 17,310 | 17,117 | 17,614 |
| National Science Foundation | 6,430 | 6,032 | 6,854 |
| Department of Energy's Office of Science | 4,398 | 3,973 | 4,722 |
| National Institute of Standards and Technology (core funding) | 594 | 519 | 634 |
| Environmental Protection Agency | 7,204 | 7,472 | 7,142 |
| National Oceanic and Atmospheric Administration | 3,815 | 3,907 | 4,110 |
| US Geological Survey | 975 | 1,006.5 | 968.5 |
| Department of Defense (basic and applied research) | 5,785 | 6,606 | 5,944 |
| Department of Homeland Security (science and technology) | 799 | 830 | 869 |

The third core agency for the competitiveness agenda, NIST, is in line to receive $634 million — 22% more than it got in 2008, with new initiatives lined up for nanotechnology and bioscience measurements.

Meanwhile, at the NIH, director Elias Zerhouni says that the agency fared well compared with many others such as the Centers for Disease Control and Prevention, which would see a $433 million, or 7%, hit. "We're very thankful in the context of this very difficult budget that we're seeing some increases and no decrease" at the NIH, Zerhouni says. (As happened last year, $300 million of the $29.3 billion agency total will not be seen by researchers, but will be transferred to the Global Fund to Fight AIDS, Tuberculosis and Malaria.)

Under Bush's proposal, the NIH would fund essentially the same number of research grants as in 2008, and keep the proportion of new grants steady. Zerhouni predicts that the success rates for competing grants, which the agency puts at 19%, "will probably stay where they are or go down a little bit". The NIH would also eliminate inflationary increases for existing grants.

Money in the budget for tiny increases at the NIH's 27 individual institutes and centres depends on eliminating $111 million for the National Children's Study, a project aiming to track influences on the health of 100,000 children from birth to age 21. The White House has repeatedly cancelled the study; Zerhouni says that the agency sees other issues as higher priorities, such as protecting investigator-initiated grants and funding for young investigators. But last year Congress reinserted the money.

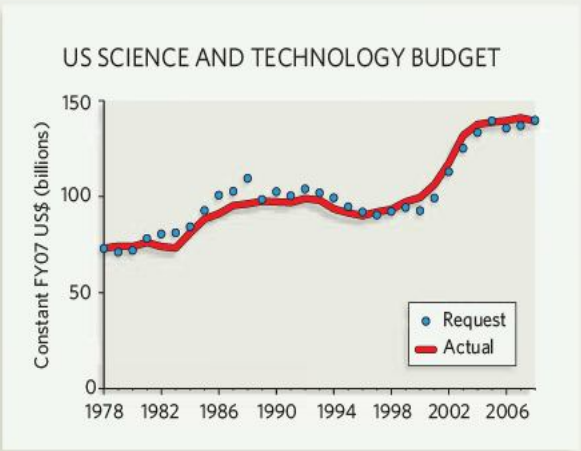
"We're looking at a continuation of a trend of essentially flat-funding for the NIH budget over the past four years," says David Moore, a senior lobbyist with the Association of American Medical Colleges in Washington DC. "When you couple that with biomedical inflation [currently put at 3.9%], we're seeing a significant erosion of this nation's medical research capacity." Using the biomedical inflation figures produced by the Department of Commerce, the request would mark the sixth straight year of level or reduced spending at the NIH. Robert Palazzo, president of the Federation of American Societies for Experimental Biology, pronounced himself "staggered" by

## Granting requests

Presidents make budget requests: Congress holds the purse strings. With the two branches of government often at odds, it may seem a wonder that the final outcome looks anything like the initial request. And yet the amount Congress spends on research and development (R&D) is usually within a few per cent of the president's request (see graphic).

This is because Congresses, cognizant of economic conditions and the limits to deficit spending, and presidents, wielding veto threats, come to broad agreement on overall levels of discretionary spending. Those, in turn, closely guide R&D support, says Kei Koizumi of the American Association for the Advancement of Science in Washington DC. Roughly one out of seven discretionary dollars goes to R&D: "In the end, all these interest groups fighting for discretionary spending fight to a draw."

Broad agreement on discretionary spending does nothing to reduce the gloating and griping of individual winners and losers. During the past few years, Congress has funded biomedical, energy and environmental research above the president's requests, while giving the physical sciences less. **E.H.**



the Bush request. "The NIH's absolute zero increase is going to strike very deeply into the heart of American research," he says.

Over at NASA, things are looking up for those who look down. The agency is budgeting to reinstate three climate sensors that had previously been removed to the pilot mission for the next generation of weather and climate monitors, the National Polar-orbiting Operational Environmental Satellite System. And following a recommendation from the US National Academies, the agency is planning to start five new Earth-observation missions in the next six years, including a satellite to measure soil moisture set for a 2012 launch, and a laser altimeter to measure ice thickness in 2015. Three other new missions, as prioritized by the academies' report, will be announced soon, at a cost of $910 million over five years.

NASA's budget request also includes $344 million in the next five years for three small lunar missions to be launched by 2014 — a dust monitor and two geophysical stations for the Moon's poles that will form part of an international network. Asked whether the new lunar science represented a renewed commitment to Bush's vision of future Moon exploration, NASA associate administrator for science Alan Stern said: "You could say, empirically, it does."

Finally, NASA announced that work would begin on the next major astrophysics flagship launch: the Joint Dark Energy Mission. The project, also funded by the DOE, was ranked first by a recent National Academies report and may fly by 2015. ■

**Eric Hand, Meredith Wadman, Rachel Courtland, Mitch Waldrop and Jeff Tollefson**

# The Moon: destination or distraction?

A high-level meeting next week will offer scientists a chance to re-examine NASA's commitment to human exploration of the Moon. The 12 February workshop is organized by the Planetary Society, a space-exploration advocacy group based in Pasadena, California. It is timed to come four years after President George W. Bush called for a return to the Moon in his Vision for Space Exploration (VSE), and a week after the last of the budget requests with which he might have furthered that vision (see page 610). As such, it might thus mark the opening of the post-Bush era in space exploration.

Conceived in the wake of the Space Shuttle Columbia disaster, the VSE's goals were to finish the International Space Station (ISS), replace the shuttle, return crews to the Moon, and eventually explore Mars. But the expense of shuttle operations and ISS construction has led to cuts in the VSE's budget, as well as in that for space science. "The Vision for Space Exploration doesn't have enough public support to generate the budget it needs," says Planetary Society director Louis Friedman. "We have an adequate window to discuss whether the lunar programme has been constructed correctly."

**"We have a window to discuss whether the lunar programme has been constructed correctly."**

On the list to attend the two-day, invitation-only meeting at Stanford University in California are 50 prestigious figures including astronauts, former aerospace-industry chief executives, a handful of former NASA associate administrators and, most importantly perhaps, the advisers to two of the presidential candidates.

Given the budgetary constraints, some of the participants want alternatives to Moon missions. One target would be the near-Earth asteroids, some of which are within the range of the Ares 1 rocket that is under development for the VSE. Because of the asteroids' low gravity, they could be landed on with a slightly modified version of the Crew Exploration Vehicle, being developed as a replacement for the Space Shuttle. Other proposals would take a crew not to natural targets but to artificial ones, such as the James Webb Space Telescope, an infrared replacement for the Hubble telescope that will orbit considerably farther from Earth. These missions would offer a chance to practise the long trips required for interplanetary travel without incurring the costs of lunar landings.

Some see a subtext here — a desire to avoid building expensive lunar infrastructure and instead focus on something more exciting. "The real reason Mars advocates like asteroids is because we aren't going to build a base on an asteroid," says James Muncy, a space-policy consultant and former adviser to the Reagan and current Bush administrations. The Planetary Society has long pushed for Mars missions,

# Carbon burial buried

**The US Department of Energy has pulled out of a flagship project to build the first 'clean' coal-fired power plant in the United States, a move that will kill the project unless supporters can rouse Congress on its behalf.**

**The FutureGen project was intended to demonstrate technologies for capturing and burying carbon dioxide from coal-fuelled power plants; it was scheduled to begin operating in 2012. But its costs have nearly doubled to $1.8 billion in recent years, and last week the department pulled out of the deal after failing to reach a new funding agreement with its private partner, the FutureGen Industrial Alliance, which consists of more than a dozen energy companies. The energy department had been slated to pick up three-quarters of the bill for the 275-megawatt plant.**

**"I'm disappointed because I thought there was a lot more good than bad in the project,"**

DOE

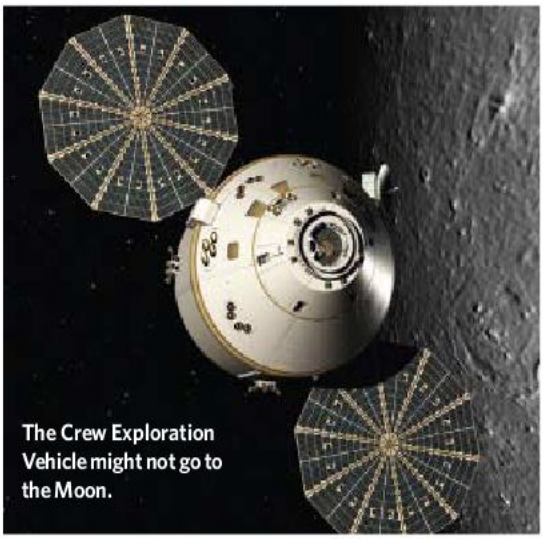
**Soaring costs mean the FutureGen power plant may never be built.**

**says Howard Herzog, a carbon-sequestration expert at the Massachusetts Institute of Technology in Cambridge. "It's hard for me to see this not delaying overall progress."**

**In the project's place, the administration says it will help companies add carbon-capture and -sequestration equipment to new or existing coal plants that have at least 300 megawatts of capacity. Officials say this will ultimately save taxpayers money while allowing the technology to spread more quickly.**

**The abrupt decision has infuriated members of the FutureGen alliance and the project's political supporters**

LOCKHEED MARTIN



The Crew Exploration Vehicle might not go to the Moon.

and one of the meeting's conveners is Stanford professor G. Scott Hubbard, a former head of the NASA Mars programme. Hubbard says that, although he personally wants to speed up Mars exploration, there is no preconceived result for the workshop. But Mike Griffin, the NASA administrator, says in an e-mail to *Nature* that some of the workshop organizers had a long-standing rejection of the Moon as a place to explore. "Balanced choices must be made," Griffin says. "But they cannot be continually remade if there is to be progress."

A new administration, though, does offer a chance to make new policy — and to appoint new administrators. "This is absolutely the season for these things," says Muncy. Lon Levin and Lori Garver, who are space-policy consultants and advisers to Democrat presidential-hopefuls Barack Obama and Hillary Clinton, are the sort of attendees who might feed the results of the workshop into new policy. Hubbard says that the exclusion of advisers to major Republican candidates, Senator John McCain (who has been endorsed by Sean O'Keefe, the former NASA administrator who launched the VSE) and former Massachusetts Governor Mitt Romney, was not intentional. Rather the Democratic advisers were included incidentally, as the Planetary Society is one of Garver's clients and Levin is a member of its board.

The Stanford workshop group is just one of many fighting for the attention of such people. "Theirs will be one more report added to dozens of other inputs that these transition teams are going to get," says Alan Ladwig, a former NASA associate administrator who supports the current programme. "Unless one has the private number of a presidential candidate, I can't imagine that it will have all that much of an impact."

But the Stanford group has a precedent. After Bush issued the skeletal version of the VSE in January 2004, many groups followed up with reports on how best to implement it. In the summer of 2004, Griffin, then at Johns Hopkins University's Applied Physics Laboratory in Laurel, Maryland, and Owen Garriott, a former astronaut, produced their own report on the VSE, commissioned by the Planetary Society. They shopped the report around the White House and Congress, where it was received favourably. By April of 2005, Griffin was NASA administrator and his report was, for the most part, subsequently implemented. "I'm sure that some of [the workshop attendees] would hope to get on one of the transition teams or get some kind of political appointment or return to NASA as a result of their activity," says Ladwig. ■

**Eric Hand**

on Capitol Hill. As recently as December, when the alliance announced that the plant would be located in Mattoon, Illinois, the energy department called the project a "cornerstone" of the administration's vision for clean coal. But deputy energy secretary Clay Sell says he realized FutureGen was in trouble when the cost estimate came out nine months before that. "I knew that this would not end well when I saw that baseline increase so dramatically this early in the process," he says.

Although the energy department and the alliance had agreed to split any further cost overruns evenly, Sell says the department objected when the alliance insisted

**"There was a lot more good than bad in the project."**

on financing its share of the project by taking out a loan — essentially reducing its cash contribution.

Rising prices for plant components such as steel and concrete, as well as labour, pushed the price tag from $950 million to $1.5 billion, says Michael Mudd, the alliance's chief executive. He points out that the additional $300 million that boosts total costs to $1.8 billion represents operating costs that will be recovered through electricity sales.

"We are going to work with Congress to make sure that the legislative language exists to keep it viable and on track as is," Mudd says. ■

Jeff Tollefson

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Additional information on the duties or any other aspect concerning the position can be found at http://www.cnethpc.es. For any question or request, please send an e-mail to sgpitgi@mec.es and cneh2pc@jccm.es

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# VICE PRESIDENT DISCOVERY (RESEARCH) — BC CANCER AGENCY
# ASSOCIATE DEAN, RESEARCH — UNIVERSITY OF BRITISH COLUMBIA

## VANCOUVER, BRITISH COLUMBIA, CANADA

**The BC Cancer Agency (BCCA)** is committed to reducing the incidence of cancer, reducing the mortality from cancer, and improving the quality of life of those living with cancer. It provides a comprehensive cancer control program for the people of British Columbia by working with community partners to deliver a range of services, including prevention, early detection, diagnosis and treatment, research, education, supportive care, rehabilitation and palliative care. It operates four regional cancer centres in the Fraser Valley, Kelowna, Vancouver and Vancouver Island, a fifth centre is opening in Abbotsford in the summer, 2008, with a sixth centre scheduled to open in Prince George in 2012. In close association with the BC Cancer Agency's Research Centre, the regional centres conduct research into the causes and cures for cancer.

BCCA is an agency of the **Provincial Health Services Authority (PHSA)** which plans, manages and evaluates specialty and province-wide health care services across BC. ***PHSA embodies values that reflect a commitment to excellence. These include: Patients first • Best value • Results matter • Improvements through knowledge • Open to possibilities.***

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The BC Cancer Agency is seeking a research leader of international profile with a record of scientific excellence to lead the 'discovery' research activities of the BC Cancer Agency. The appointment will also serve as Associate Dean, Research within the Faculty of Medicine at the University of British Columbia. The BC Cancer Agency is affiliated with the University of BC and co-hosts the development and conduct of academic oncology in BC. The position entails responsibility for visionary, strategic leadership and overall direction, coordination and oversight of 'discovery' cancer research at the BC Cancer Agency. The Discovery Research portfolio comprises 60 principal scientists and a total of 640 scientific and medical personnel organized within 10 research divisions. (Advanced Therapeutics, the Michael Smith Genome Sciences Centre, Cancer Control Research, Cancer Endocrinology, Cancer Genetics and Developmental Biology, Cancer Imaging, Medical Biophysics, the Terry Fox Laboratory, the Breast Cancer Research Chair program, and the Deeley Research Centre located in Victoria).

The BC Cancer Agency's Research Centre is one of the largest free-standing cancer research facilities in Canada, occupying 231,000 sq ft (Vancouver) and 18,000 sq ft (Victoria). Current research funding exceeds $60M per annum.

The University of British Columbia is Canada's third largest university and consistently ranks among the 40 best universities in the world. Primarily situated in Vancouver, UBC is a research-intensive university and has an economic impact of $4 billion to the provincial economy. The Faculty of Medicine at UBC, together with its partners including B.C.'s Health Authorities, provides innovative programs in the areas of health and life sciences through a province-wide delivery model. The Faculty teaches students at the undergraduate, graduate and postgraduate levels and generates more than $200 million in research funding each year. It is home to Canada's first distributed MD undergraduate program.

Applications are invited from individuals who hold PhD and/or MD qualifications. In addition, they should be eligible for appointment at the Associate or Full Professor level at University of British Columbia, and, if medically qualified and wishing to practice clinical medicine, hold or be eligible for Canadian specialist qualifications in the appropriate discipline and be eligible for licensure to practice medicine in BC. Key attributes of the successful applicant will include scientific excellence, innovation, collaborative relationships with local, national and international partners, promotion of 'team research' and the ability to lead a strategically focused program of excellence in scientific discovery.

A letter of application should be submitted with a current curriculum vitae to: **Stephanie Milliken - smilliken@telus.net** The closing date for applications is **March 10th, 2008.**

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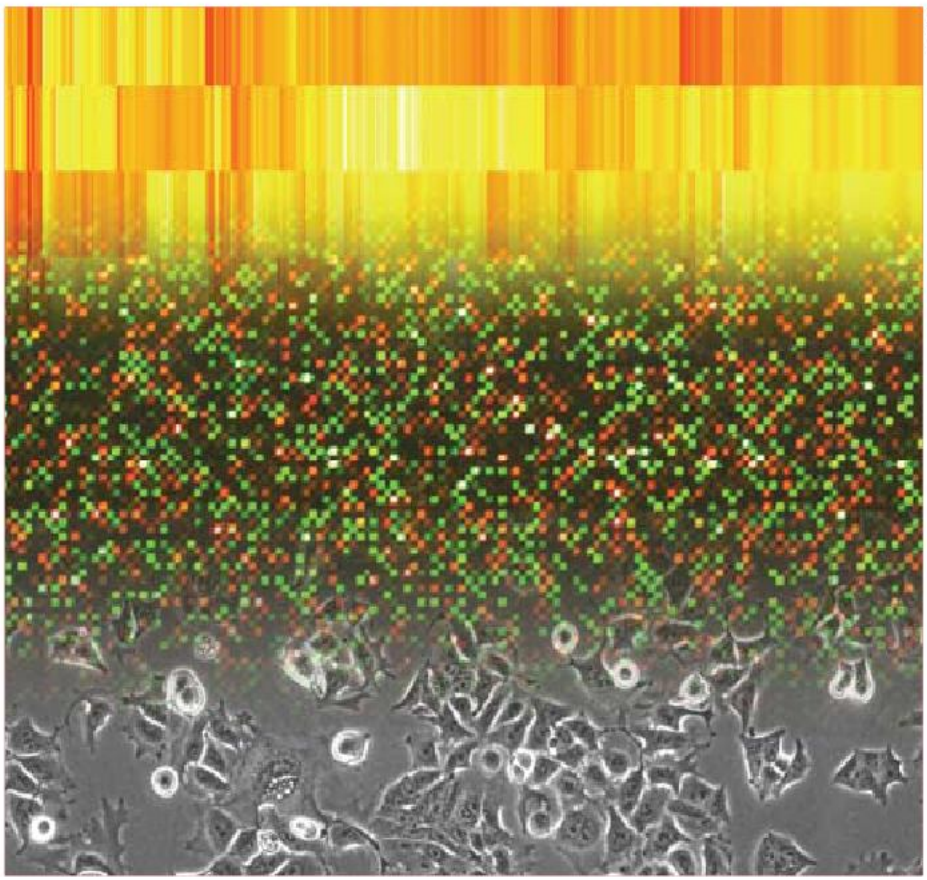
# 'Normal' genes key to cancer growth

Geneticists have identified genes that are normally present and that seem to be key to the growth and survival of specific cancers. The finding, from a 'functional-genomics' screen of human cells, could offer new drug targets for blitzing tumours.

In an alternative approach to the traditional search for oncogenes (rogue genes that can turn normal cells into tumours), two teams of US scientists publish support this week for what they call the "non-oncogene addiction" idea: that a tumour relies heavily on certain normal cell pathways, and that drugs disabling gene products in those pathways could be deadly to cancer. The teams, led by Stephen Elledge of Brigham and Women's Hospital in Boston, Massachusetts, and Greg Hannon of Cold Spring Harbor Laboratory in New York, designed a method to knock down thousands of genes relatively cheaply and quickly.

The method uses 'short hairpin RNAs' (shRNAs) — pieces of RNA that can be designed to target and shut off specific genes. In two papers[1,2], the teams describe how they introduced thousands of shRNAs that target normal genes into colon cancer cells, breast cancer cells and healthy breast cells. Dozens of the shRNAs slowed or stopped the cancer cells from growing, but didn't impair the healthy cells, which could point the way to new cancer drug targets.



SCIENCE

Gene signatures (top) result from multi-gene analysis (middle) of cancer-cell survival (bottom).

"It will take time and money to sort out which of these are the best drug targets, but the important thing is that we are finding them," Elledge says.

Oncologists say that drugs against oncogene products, such as Novartis' Gleevec (imatinib) and Genentech's Tarceva (erlotinib), have been a welcome advance for patients with cancer. But because patients often develop resistance to the drugs, they are not cures, says Gary Schwartz of New York's Memorial Sloan-Kettering Cancer Center. "Everyone's looking for the next Gleevec, but even though these targeted drugs have been exciting, they have not had the overwhelming impact we would have hoped for," says Schwartz, who is trying to find funding for a clinical trial of a drug that inhibits a normal cell-cycle pathway[3]. Early clinical trials of the drug indicate that it may operate best in a 'therapeutic window' in which it is more harmful to cancer cells than to healthy cells, Schwartz says.

Elledge and Hannon say that their work will complement two large projects aiming to spur development of more drugs like Gleevec and Tarceva, which target mutations involved in certain blood and lung cancers. Both projects involve sequencing the genomes of cancer cells to find more oncogenes and help scientists understand the biology of cancer. One — the Cancer Genome Atlas — began in December 2005, and is expected to cost $1.35 billion over 9 years. The other is led by scientists at the Wellcome Trust Sanger Institute in Cambridge, UK.

**"The sequencing becomes the easy part — the longer road is going to be to figure out what it all means in functional studies."**

Both Elledge and Hannon have long contended that the sequencing studies have a low "bang for the buck" — they are quite costly and will require detailed follow-up studies to sort out the mutations that drive cancers from those that are merely along for the ride. Their work is, by contrast, cheap and easy enough to be done in a single laboratory. And they that say their "functional" approach, which looks at the behaviour of cancer cells in response to certain triggers, might be a quicker path to new drugs.

Elledge and Hannon have found growing support for their argument, even among scientists using the sequencing approach. Bert Vogelstein, co-director of the Ludwig Center at Johns Hopkins School of Medicine in Baltimore, Maryland, says that his own studies highlight the need for the cancer atlas to fund some functional-genomics work. "What's clear now that wasn't clear at the beginning of the Cancer Genome Atlas is the complexity and heterogeneity of the mutational signatures that are going to be found in most cancers," says Vogelstein, who demonstrated such complexity in a 2006 study on breast and colorectal cancers[4].

"The sequencing becomes the easy part — the harder and longer road is going to be to figure out what it all means in functional studies," Vogelstein says. "I believe some well-thought-out combination of those two approaches is likely to be the best way to progress." ■

**Erika Check Hayden**

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3. Fornier, M. N. *et al. Clin. Cancer Res.* **13,** 5841–5846 (2007).
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## ON THE RECORD

**"Richard Dawkins has proven to be one of the most wicked human beings to ever walk the earth."**

Hardline Creationists behind the website http://preachingyourfuneral.com attempt to justify their decision to hold a mock funeral for their ideological enemy.

## NUMBER CRUNCH

**4** is the number of Internet cables damaged in a spate of incidents that has left much of the Middle East and India without telecommunications.

**100%** is the reported loss of Internet connection in Iran. Israel and Iraq were unaffected, leading some bloggers to suspect a conspiracy ...

**1** is the number of ships accused of causing the most serious incident — it is still unclear whether an errant anchor off the Egyptian coast accidentally cut the first two cables.

## SHOWBIZ NEWS

**Back to bleak**

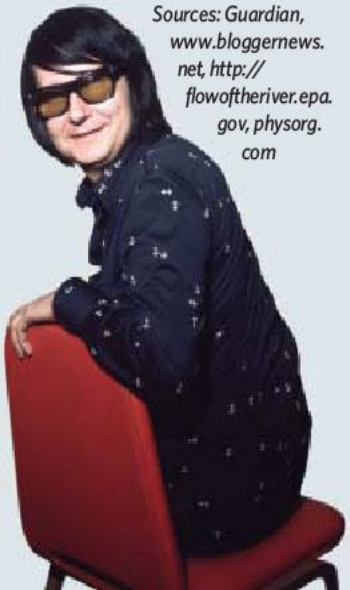
Deputy administrator of the US Environmental Protection Agency Marcus Peacock raised eyebrows by using his official blog to draw the all-too-obvious parallels between environmental degradation and the much-publicized drug addiction of British singer Amy Winehouse.

## ZOO NEWS

**Beetle's new release**

Staying with singers, the late, great Roy Orbison has received a new accolade — he's the inspiration behind the name of the newly discovered whirligig beetle *Orectochilus orbisonorum*.

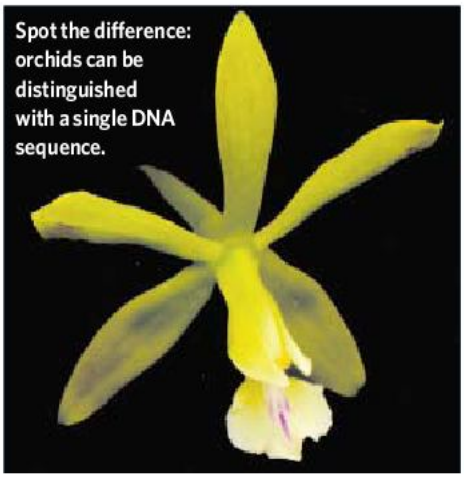
P. FIEVEZ/BIPS/GETTY IMAGES



*Sources: Guardian, www.bloggernews.net, http://flowoftheriver.epa.gov, physorg.com*

SIDELINES

D. BOGARIN



F. PUPULIN

Spot the difference: orchids can be distinguished with a single DNA sequence.

# Botanical identities

Researchers have used a DNA sequence to distinguish between more than 1,600 botanical samples from two biodiversity hotspots, providing the largest test yet of 'DNA barcoding' in plants.

But this will not end the ongoing debate over which barcodes botanists should adopt. "I think this is a step forward," says John Kress of the Smithsonian Institution's National Museum of Natural History in Washington DC. "But I don't think it means we're there yet."

DNA barcodes are sequences that vary extensively between species but hardly at all within them, and so can be used to distinguish one species from another. Established barcodes could be used to quickly inventory biodiversity in a protected area, for example, or to monitor shipments of plants for illegal trading of endangered species.

Establishing a barcode for animals has been fairly easy; part of a gene called *CO1*, which has been used for years to study animal family trees, fulfils the requirements well. But plants have been more problematic. Labs around the world have churned out paper after paper supporting various alternatives to *CO1*. Each lab designed its experiments differently and tested its barcodes on different sets of plants. Some moved ahead on large-scale projects using their favoured barcodes regardless of the field's lack of consensus.

"It's a very contentious issue," says Kenneth Cameron, director of the Wisconsin State Herbarium at the University of Wisconsin-Madison. "There are a lot of politics and personalities involved."

It was against this background that Vincent Savolainen of the Royal Botanic Gardens, Kew in London decided to compare some of the leading barcode candidates across a large set of samples. As they report this week in *Proceedings of the National Academy of Sciences*, he and his colleagues tested samples from 86 species from southern Africa and Costa Rica using eight barcodes (R. Lahaye *et al. Proc. Natl Acad. Sci. USA* doi:10.1073/pnas.0709936105; 2008).

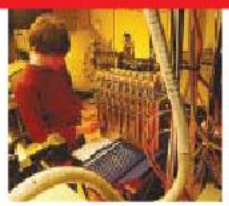
The researchers found that barcodes from genes called *matK* and *trnH-psbA*, used either alone or together, correctly classified just over 90% of the species. Because *trnH-psbA* sequences can be difficult to compare across species, they went on to inventory another 1,036 species of orchid using *matK* alone.

But this does not make *matK* the uncontested champ. For one thing, researchers have reported difficulty amplifying the gene for sequencing in some plants. Savolainen says his team used an improved amplification protocol, but Kress says the new method still fails with some species. And a better idea of the technique's range is needed: it may do well with orchids, but what of liverworts or ferns?

Most suspect that, in the end, a single barcode will not suffice. At a meeting of the Consortium for the Barcode of Life in Taipei, Taiwan, last autumn, the Plant Working Group proposed three barcodes: *matK*, *trnH-psbA* and another called *atpF-H*. Savolainen and his team think *matk* and *trnH-psbA* may suffice.

The next step will be a test of all the leading barcodes against 675 species of plants. This test is being coordinated by Peter Hollingsworth of the Royal Botanic Gardens in Edinburgh, UK, who runs the barcode consortium's Plant Working Group. It will study the reproducibility of results in different labs, with the outcome expected in April. Kress is optimistic that this may lead to a consensus, but acknowledges that the delays and bickering have been frustrating. "I'm beginning to think I'm going to start working on fruitflies," he jokes. ■

**Heidi Ledford**



J. KING-HOLMES/SPL

**UNRAVELLING CARBON'S SECRETS**
Nuclear theory aims to explain why carbon dating works.
www.nature.com/news

# 'Monogamous' vole in love-rat shock

By traditionalist standards, prairie-vole couples may enjoy the ideal relationship: the rodents form lifelong partnerships — a highly unusual practice in mammals. Males help raise the children; females help build the nest. As for their sex life, let's just say it far exceeds the efforts required for procreation.

But the respectable public behaviour of North American prairie voles (*Microtus ochrogaster*) may hide a bed-hopping double life. Paternity tests published last week indicate that the animals touted as paragons of monogamy frequently cheat on their partners (A. G. Ophir *et al. Anim. Behav.* doi:10.1016/j.anbehav.2007.09.022; 2008). "Ironically", the study's authors conclude, "the dissociation of social and sexual fidelity leads us to suggest that prairie voles are even better models of human attachment than has been appreciated."

Studies on prairie voles have led scientists to look at the role of hormones such as vasopressin and oxytocin in strengthening human relationships. Revelations of infidelity in the creatures will not change the significance of that research, but may make the voles a little less popular among political agitators for sexual abstinence. (Eric Keroack, who headed a government family-planning committee in the United States, even used the monogamous voles as evidence to support his view that people who have extra-marital sex damage their oxytocin signalling mechanisms.)

**"Humans want to believe in sexual monogamy."**

Over the past few decades researchers have learned to distinguish between 'social monogamy' — in which a pair lives and tends their young together — and 'sexual monogamy', in which a couple mates exclusively with each other. "You may have a partner you come home to every night," says Alexander Ophir, a biologist at the University of Florida in Gainesville, "but that's not necessarily the one that you're mating with."

Ophir and his colleagues found that infidelity had no effect on reproductive success: a cheating vole was just as likely to reproduce as a faithful one, so long as the cheater maintained a socially monogamous relationship. Sue Carter, a biologist at the University of Illinois at Chicago, says that these findings highlight the importance of social bonding. "Humans want to believe in sexual monogamy," says Carter. That focus may have distracted people from the relative importance of social monogamy, she says.

Carter has observed philandering voles in her own lab, and notes that the infidelity did not disrupt pre-existing partnerships. When a female initiates contact with an outside male, for example, the relationship remains strictly sexual. "She mated with him," says Carter, "and then she attacked him, ran him off and went back to her established partner." ■

**Heidi Ledford**

## Wellcome Trust announces spending bonanza

The Wellcome Trust, the world's largest medical research charity, is planning to boost its annual spending.

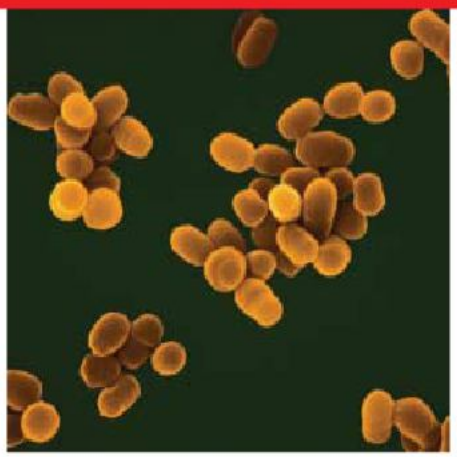
The trust, which backs a broad range of biomedical research, will increase its expenditure by 60% to £4 billion (US$8 billion) over the next five years.

Total grant funding is expected to rise from £520 million to £650 million annually, according to spokesman Craig Brierley. A portion of the new money will go towards research into the genetic basis for common diseases such as cancer and diabetes.

Some £500 million will go towards assisting construction of a new biomedical campus in London, a fund to develop new treatments based on fundamental science, and a campaign to improve Africa's biomedical infrastructure.

## Lab workers exposed to brucellosis by safety tests

More than 900 people working in 254 labs around the United States and Canada might have been exposed to modified *Brucella abortus* last autumn, because of their failure to follow proper handling procedures for the bacterium, the *Atlanta Journal-Constitution* has reported.



PHOTOTAKE/ALAMY

**Handle with care:** *Brucella abortus.*

*Brucella abortus* primarily causes disease in cows, but it can also make people ill. It was posted to 1,316 clinical laboratories in a joint exercise — led by the College of American Pathologists, the Centers for Disease Control and Prevention, and the Association of Public Health Laboratories — to test the labs' procedures for dealing with suspected bioterrorism agents.

Also last week, watchdog groups reacted with outrage to news that virologist Yoshihiro Kawaoka is working with a modified Ebola virus in a biosafety level-2 (BSL-2) laboratory at the University of Wisconsin in Madison, but has not consulted the US National Institutes of Health (NIH) about the work. The NIH previously overruled a decision by the university to allow Kawaoka to work with Ebola genes in a BSL-3 lab, saying that it wanted him in a BSL-4 one.

## No conflict of interest in misconduct case, says lab

Officials at the US Department of Energy's Office of Science are resisting a finding by department attorneys that a report of an investigation into alleged scientific misconduct at a national laboratory qualifies for public release, according to a 15 January letter from the department.

The report reviews allegations against electron-microscopy researchers in the group of Stephen Pennycook at Oak Ridge National Laboratory in Tennessee (see *Nature* 450, 590; 2007). One of the report's three authors, David Williams of the University of Alabama in Huntsville, says investigators spent hours reading papers received from Oak Ridge management, and found mistakes but no misconduct.

Williams also confirms that, prior to the investigation, he and co-editor Barry

Carter of the University of Connecticut in Storrs had invited Pennycook to contribute a chapter to a future reference book on electron microscopy, with a possibility to share royalties. Williams did not declare the interaction. Oak Ridge claims that when it found out — well after the investigation had been completed — its lawyer said that there was no conflict of interest.

## Web alliance speeds up communication with Africa

African researchers have just been connected by a high-speed Internet link to Europe's research network, GÉANT2. The deal has been struck between GÉANT2 and UbuntuNet, an alliance of African national research networks created in 2005. It will speed up communications and data transfer not only between African scientists and the estimated 30 million GÉANT2 users in 34 European countries, but also with scientists worldwide — through GÉANT2's connections with the United States and other research networks around the world.

UbuntuNet is not related to Ubuntu, the popular free version of the Linux operating system. But both namesakes help bring affordable quality information technology to African scientists.

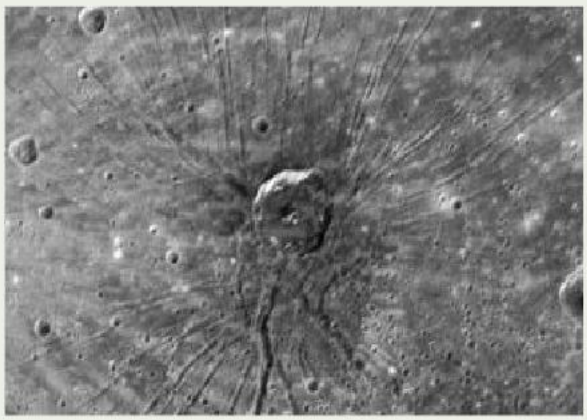
## Review article retracted amid plagiarism claims

A review article written by a rheumatologist at Harvard Medical School in Boston, Massachusetts, has been retracted after the journal, *Best Practice & Research Clinical Rheumatology*, learned that more than half of the paper may have been plagiarized.

The 2004 article, by Lee Simon (*Best Pract. Res. Clin. Rheumatol.* 18, 507–538; 2004), was manually checked after surfacing in an automated trawl through 7 million biomedical abstracts for possible plagiarism (see *Nature* 451, 397–399; 2008). The retraction was announced on 29 January.

Harvard Medical School has formed a committee to review the matter but has not launched an official investigation, says spokesman David Cameron. Simon declined to comment, saying only: "I'm very sorry that I've been so targeted for something like a review article."

## Probe catches glimpse of surface troughs on Mercury



NASA/JOHNS HOPKINS UNIV/CARNEGIE INST. WASHINGTON

Down on the floor of the immense Caloris Basin — an impact crater at least 1,300 kilometres wide that scars the surface of the planet Mercury — lies a set of radial troughs nicknamed 'the spider' (right).

This image is one of many sent back by NASA's MESSENGER spacecraft during its 14 January flyby, the first of three planned before it settles into orbit around Mercury in 2011. Project scientists think the troughs in the spider formed as material in the floor of the Caloris Basin pulled apart. A smaller impact crater near the centre may also have contributed to trough formation.

# Cell Death & Differentiation

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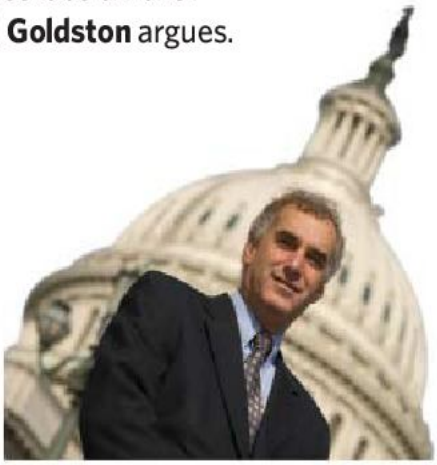
# A debatable proposition

Having the US presidential candidates face off over science issues could backfire, **David Goldston** argues.



## PARTY OF ONE

Scientists pride themselves on being independent thinkers, yet that trait isn't always apparent when it comes to politics. Take, for example, the current web-based petition to push the US presidential candidates to hold a 'science debate' (www.sciencedebate2008.com). Entranced by the notion of drawing more attention to science, prominent leaders in the community sign up almost every day with great fanfare. But no one seems to have thought through whether such a debate would actually serve the cause of science. Here are some questions the petitioners ought to be asking.

First, is it helpful to categorize a wide range of issues as matters of science? The petitioners' list of possible debate topics includes climate change, energy policy and other broad policy areas. Certainly, the presidential candidates should be compelled to talk more about climate and energy. But are these primarily science issues? Is there a scientific position on whether a carbon tax is a good idea, or how to structure one? The increasing tendency to conflate science questions — Are we experiencing man-made climate change? — with policy questions — What, if anything, should we do about it? — has been a damaging trend. It has helped to turn science into a political football and has muddied policy debates. At a 'science debate', candidates will try to claim that their position is the one supported by 'science', and the very structure of the debate will send voters the faulty message that these are questions that the natural sciences can resolve. Framing questions of economics, ethics and other aspects of policy as 'science issues' does no favour for either science or politics. And it makes one wonder if the sponsors of the debate merely want to find out whether the candidates agree with their personal opinions on these topics.

Second, is it helpful to have a high-profile debate on research spending? When asked why a debate is needed, petition sponsors often cite the need for greater research spending. The premise here seems to be that the drive to double the budget of the National Institutes of Health (NIH) got a boost when NIH funding became an issue in presidential elections, so the same strategy ought to be used for the physical sciences. But the NIH story should actually give advocates pause. Many scientists believe that the doubling between 1998 and 2003 was mishandled, leaving the field with too many new facilities and too few new researchers. And once the doubling was over, NIH funding came to a standstill. Arguably, both these problems were aggravated, if not caused, by raising the political profile of the NIH. The extra attention made the doubling seem like a one-time presidential initiative that could not be evaluated or slowed. Moreover, the NIH budget would probably have seen healthy (and perhaps more sustainable) increases without having been injected into presidential politics. The key proponents of the funding were appropriators in Congress who were already making headway before the idea had the imprimatur of the White House.

And more attention doesn't always translate into more money. The National Science Foundation (NSF) budget has fared relatively well over the years even though the agency is not particularly well known. The NSF's headaches have come when it has been in the political spotlight, as politicians raised questions about the legitimacy of specific grants. In contrast to the NSF, NASA is a household word, but its budget is a political football, and public attitudes toward the agency are mercurial and ambivalent. The debate proposal presumes that to know science agencies is to love them, but that is not borne out by history. In any event, the best indicator of how science will fare under a president is what the candidate says not about science, but rather about domestic spending (see *Nature* **449**, 962; 2007).

Third, is a political debate the best place to discuss the 'politicization' of science? 'Scientific integrity in government' is the other topic that seems to be prompting calls for the debate. But this may be a prime example of an important issue that a debate is not well structured to handle. All issues have subtleties, but this whole question requires a fine-grained discussion of what's science, what's policy, what kinds of work constitute a conflict of interest, and so on. It would be great if all the candidates pledged to appoint scientific advisers on the basis of merit and not to silence government scientists. But 'merit' can mean many things, and the rough-and-tumble of a presidential debate is an unlikely place to sort them out. In fact, if the candidates went beyond anodyne commitments, a debate could easily leave the public even more confused about how a president should use scientific advice. After all, science rarely presents presidents with a clear consensus.

In short, there is no reason to assume that a presidential debate on science matters would be instructive for the public or helpful to scientists. Indeed, have any of the debates thus far done much to clarify policy details? They are much better at underscoring stylistic and broad symbolic differences between the candidates — hardly what the 'science debate' advocates seem to have in mind. Has anyone thought through what a 'science debate' would sound like?

For example, would a debate about teaching evolution help the public to understand the science of evolution more clearly? Would the interest of science be served by nationalizing that debate? One leading scientist recently argued that a discussion of evolution is necessary because a politician who doesn't believe in evolution won't heed scientists on any other issue either, but this is demonstrably untrue. Indeed, a disbelief in evolution, distressing as that is, doesn't even lead politicians to question research spending in biology.

The rush to sign the petition may signal scientists' newfound faith in democracy, but it is more likely to reflect some old misconceptions — that scientists are being slighted if their concerns are not in the limelight, that a high political profile is always the road to success, that anyone with scientific information will arrive at the same policy conclusions. If scientists want to help their cause, they might be better served spending their time on lobbying Capitol Hill and talking to candidates — the kind of political activity often seen as 'dirty work' — rather than leaping into the showy realm of presidential debates. Without further analysis, the idea that a debate will propel the cause of science is more magical thinking than scientific. ■

**David Goldston is a visiting lecturer in science policy at Harvard University in Cambridge, Massachusetts. Reach him at partyofonecolumn@gmail.com.**

**See Editorial, page 605.**

**Editor's note: This column will now appear in the first issue of every month.**

# STUCK IN NEW JERSEY

Scientists and politicians in New Jersey thought that they had a chance to make their state a stem-cell player. Voters thought otherwise. As proponents prepare for a second attempt, **Meredith Wadman** investigates what went wrong in the Garden State.

In the middle of downtown New Brunswick, New Jersey, a small dirt parking lot lies nestled between the state's medical school, the university hospital and the Cancer Institute of New Jersey. It's not much to look at. But Wise Young sees something else: The Stem Cell Institute of New Jersey — a 14-storey tower dedicated to cutting-edge stem-cell research of the sort that Young, a highly regarded neuroscientist at nearby Rutgers University, says is vital to the future of medicine.

> "The eyes of the world are watching New Jersey."
> — Wise Young

The tower is more than just a figment of Young's imagination. Plans have been drawn up; the money to build it has been authorized; and the governor, Jon Corzine (Democrat), posed for cameras at the formal ground-breaking ceremony last October. But ceremony has given way to quiet reality; the tower has little purpose if there is no money to fund research within it.

Young has dedicated years to trying to get that money. In 2007, he was one of the leaders of a group trying to convince New Jersey's citizens to borrow almost half a billion dollars to finance research in the tower and at other facilities. The funding required a public vote, and the polls indicated that New Jersey was game. Nevertheless, Young and his colleagues were trounced: outmanoeuvred and out-marketed by two nimble political action groups.

In November, Young wants to go into battle again. Otherwise, he says, New Jersey will fall behind other states in stem-cell research. But first he and his colleagues need to learn from the failures of their last campaign, which have left the future that he dreams of stuck in a down-at-heel parking lot.

Several US states have launched campaigns against the national policy that restricts federal funding to work on the 20 or so stem-cell lines derived before 9 August, 2001. New Jersey was the first to appropriate state funding for stem-cell research, approving US$10 million in January 2004. In November of that year, the move was eclipsed when voters in California approved Proposition 71, authorizing $3 billion in state borrowing to fund stem-cell research for 10 years. Encouraged by California's success, other states followed, among them New York, where the state government last year established a $600-million stem-cell research fund, and Maryland, which has established a commission to dole out $38 million. Wisconsin is set to spend $750 million on research facilities. And last year, Massachusetts governor Deval Patrick proposed $1 billion in state funding for biomedical research — half of which would be used to establish a research centre that would house the nation's largest embryonic stem-cell bank. But not surprisingly in a country with stark political divides from state to state, many have opposed the research (see 'The state funding scrum'). Six states have criminalized it. Colorado voters may weigh in this November on a referendum declaring that legal 'personhood' begins at the moment of conception.

A year ago, observers could have been forgiven for thinking that New Jersey had a good chance of boosting its own stem-cell coffers. In addition to being the first to fund the research, it's a liberal state with a governor who is highly

## THE LONG TRAIL

For more than four years, New Jersey has been a battleground for advocates and opponents of using state money to fund human embryonic stem-cell research.

### 4 Jan 2004

New Jersey governor James McGreevey signs a bill legalizing embryonic stem-cell research in the state (above). He is joined by the late Christopher Reeve, an actor who lobbied for the bill.

### 9 Feb 2004

Wise Young (centre), an advocate for spinal-cord research writes to McGreevey asking for funding for stem-cell research.

### 20 June 2005

Marie Tasy (above), of New Jersey Right To Life, testifies against a proposed bill to fund human embryonic stem-cell research.

S. CHERNIN/GETTY IMAGES; N. ROMANENKO/RUTGERS UNIV.; N. ADDIS/STAR-LEDGER

supportive of the measure and major population centres that are effectively suburbs of Manhattan and Philadelphia. The 'Garden State' is home to 17 of the world's biggest pharmaceutical companies, and research and development and biotech firms are blossoming up and down the New Jersey Turnpike, the toll road that runs the length of the state. But as in California, advocates such as Young also faced challenges to their grand vision. Some were predictable, such as the state's $32-billion debt and its looming $3-billion budget deficit. Others they should have anticipated — the short campaign season, the off-year election and fiercely committed opponents who were able to rally major grass-roots support to influence the media and win the election.

## Medical innovator

Young, 58, was born in Hong Kong and grew up in Japan. After getting an MD at Stanford University in California and a doctorate in physiology and biophysics from the University of Iowa, he started a neurosurgery residency at New York University Medical Center. But after Young had to tell the parents of a 17-year-old wrestler that their son would never walk again, he quit the residency for full-time spinal-cord research. By the time he was 40, he and his collaborators had upended conventional wisdom on the irreversibility of spinal-cord injury by showing that high doses of the steroid methylprednisolone could save about 20% of a victim's function if given within 8 hours of injury (M. Bracken *et al. N. Engl. J. Med.* **322,** 1405–1411; 1990). Young became a hero to legions of people in wheelchairs, including Christopher Reeve, the former actor and stem-cell research advocate, who sought Young out as an adviser and confidant after he was paralysed in a riding accident in 1995. Two years later, Rutgers University wooed Young and made him the founding director of the W. M. Keck Center for Collaborative Neuroscience, and its patient-outreach arm, The Spinal Cord Injury Project.

Then, in 1998, scientists managed to isolate human embryonic stem cells. Young saw the potential of the cells for spinal-cord-injury research. "It opened new possibilities we hadn't realized before," he says. He was therefore thrilled when, early in 2004, New Jersey's then-governor James McGreevey (Democrat) signed a law permitting human embryonic stem-cell research and somatic-cell nuclear transfer in the state of New Jersey.


**The groundbreaking ceremony for New Jersey's stem-cell institute might have been premature.**

But that wasn't enough for Young. Days after the law was signed, he wrote to McGreevey proposing that the state fund a $50-million bond initiative to establish a state-financed stem-cell institute. "The eyes of the world are watching New Jersey," he wrote. "It would be a shame if stem-cell research in the state does not advance."

Nine months later, Reeve, who grew up in Princeton, died at the age of 52. Young was devastated. "It was the fact that he died without seeing what he fought for come to fruition — that was the saddest part," he says. "It was Christopher's death that really galvanized me."

By December 2006, when the new governor Jon Corzine signed a bill into law to establish several stem-cell research facilities in New Jersey, the $50 million in bonds that Young had proposed to McGreevey had grown to $270 million. Of this, $150 million would build the Stem Cell Research Institute of New Jersey, run jointly by Rutgers and the University of Medicine and Dentistry of New Jersey's Robert Wood Johnson Medical School.

As 2007 started, all that remained for Young and his allies was to find funding for the research that would fill that brand-new building. After a protracted battle in the state legislature, Jersey lawmakers passed a third bill in June, the New Jersey Stem Cell Research Bond Act. It proposed a nearly half-billion-dollar loan that the voters would have to approve. The ballot question, as laid out in the bill, asked for permission for the state to borrow $450 million over 10 years to fund stem-cell research.

In an accompanying "interpretive" statement that voters would read on election day, ballot question two noted that the loan could benefit New Jersey residents "with diseases and severe injuries such as Alzheimer's disease, cancer, diabetes, Lou Gehrig's disease, Parkinson's disease, sickle-cell anaemia and spinal-cord injuries."

Ballot question two didn't mention higher taxes explicitly; it asked voters to approve both the loan and unspecified "ways and means" for the state to pay back the capital and interest. But buried in the 17-page bill that created the bond question was a sentence that caught the notice of Steve Lonegan, founder of the New Jersey branch of Americans for Prosperity, an anti-tax

N. ROMANENKO/RUTGERS UNIV.

J. O'BOYLE/STAR-LEDGER/CORBIS; AMERICANS FOR PROSPERITY; M. DERER/AP

## 20 Dec 2006

Governor Jon Corzine (below) signs a bill into law authorizing the state to borrow $270 million for stem-cell research facilities.

## 2 Oct 2007

Steve Lonegan (below), head of the local branch of Americans for Prosperity, organizes rallies (centre) against question two and other ballot measures. The money starts to pour in.


## 26 July 2007

Corzine (above) signs the Stem Cell Research Bond Act, putting 'ballot question two' to the public and asking if the state can borrow another $450 million to fund the research.

group with national headquarters in Washington DC. The sentence that leaped out at him said that if the state lacked the funds to pay back the interest and capital on the bonds — payments that government estimates put as high as $37 million per year at their peak — it must tax "the real and personal property" of New Jerseyans to make up the deficit.

In a state with some of the highest property taxes in the nation, Lonegan knew a target when he saw one. So, from his office in Bogota, a gritty town at the north end of the Jersey Turnpike, Lonegan spent his summer raising cash.

Lonegan collected some $450,000, all of it, he says, from unnamed New Jersey donors, who were also motivated to fight two other ballot questions that Lonegan's group was opposing — on sales taxes and borrowing to preserve open space. The bulk of the money poured into his office between 2 October and 6 November. In a media market in which television time can cost as much as $30,000 per 30 seconds, and a day of radio airtime tens of thousands of dollars, money was crucial.

> "Ballot question two was a very deceptive measure."
> — Marie Tasy

## Fighting back

An hour south on the turnpike, Marie Tasy was also preparing for battle. As executive director of New Jersey Right to Life, Tasy is a political force to be reckoned with. She had been fighting stem-cell research since it first found its way onto the political agenda. Tasy was furious, in particular about the wording of the ballot question. It did not spell out that stem-cell research included research on human embryonic stem cells. She was equally incensed that it didn't point out that, under New Jersey's 2004 law, the funds could be used to clone human embryos from which to harvest stem cells, or even — in her interpretation of the law's wording, which has not been tested in court — to implant cloned embryos in a womb for gestation.

"It was a very deceptive measure," says Tasy, a well-kept woman with huge brown eyes and a direct gaze, who keeps a portrait of a beatific Mother Teresa praying the rosary and a picture of herself with President George W. Bush in her Piscataway office. "It did not specify what type of stem-cell research would be performed. It did not mention that cloning would be involved up to the point of the fetal stage. It did not mention how the funding for the bonding was going to be paid back."

On 18 September, New Jersey Right to Life sued the Corzine administration in the state's superior court, arguing that the referendum should be stopped because of this "deceptive" wording. The final decision allowing the vote to commence wouldn't arrive until 26 October, 12 days before the election.

Meanwhile, Tasy came up with a killer nickname for ballot question two: "Loan to clone" — a media-friendly sound bite that she repeated at every opportunity. She also hired Rick Shaftan, a conservative media consultant. On 19 October, he helped produce a television advertisement featuring Steven McDonald, a Long Island police detective who was shot and paralysed in the line of duty in 1986. In it, the wheelchair-bound detective, in front of a US flag, declares earnestly that "question two is about taking your tax dollars for something that Wall Street and the drug companies won't invest in. Think about it."

New Jersey Right to Life reports $7,500 in media expenditures for the period, but Shaftan says that $50,000–75,000 would be a reasonable estimate of the group's total spending on the campaign. During an in-person interview, Tasy declined to specify what her group spent, and she did not respond to later requests for further information.

## Campaign trail

In late July, thirteen miles east of Tasy's headquarters, Young and his pro-stem-cell allies first met around a conference table at the law firm of Wilentz, Goldman & Spitzer, which occupies five floors of a ten-storey office building in Woodbridge. There, Ed Albowicz, a 32-year-old associate reputed to have the tenacity of a bulldog, had been signed off by his bosses to work on behalf of the stem-cell cause. Albowicz's mother had recently died of ovarian cancer, and he was convinced that the research, given a chance, could help save others like her. The firm's octogenarian senior partner Warren Wilentz still visits the office in the wheelchair he's been confined to since a car accident several years ago.

The meetings became a weekly event, attended by ten or so people including Young, Albowicz and Russ Oster, a Pennsylvania-based Democratic political operative and direct-mail specialist who had been involved in dozens of campaigns. Young and his allies had reason to be confident: earlier that month, a poll done by Quinnipiac University in Hamden, Connecticut, had reported that 71% of New Jersey voters supported embryonic stem-cell research; 19% opposed it. But there was also cause for concern: that support softened to 49%, and opposition rose to 39%, when people were asked whether New Jersey should finance the research.

Oster, for his part, remembers the summer as a time when stem-cell proponents "were talking among themselves, saying: 'We gotta do something. What do we do?'" He was especially worried by that 49% support figure: "Any time you start a campaign where you're trying to get


STAR-LEDGER; N. ROMANENKO/RUTGERS UNIV.

**7 Oct 2007**

All five Roman Catholic bishops in New Jersey appealed to their parishes to pray against ballot question two.

**21 Oct 2007**

An editorial in New Jersey's newspaper, the *Star-Ledger* asks voters whether the state should be put further in debt.

**23 Oct 2007**

Groundbreaking ceremony for the Stem Cell Institute of New Jersey, in New Brunswick with the mother and brother of Christopher Reeve.

people to vote 'yes' and you're under 50%, it's a warning sign," he says. "It was pretty evident to me that this was definitely going to be a fight."

They began to map out a strategy: they would aim to run television ads for at least two weeks across the state; be in people's mailboxes twice before the election; do phone outreach by harnessing groups like the Juvenile Diabetes Research Foundation and the Parkinson Alliance. They would need, they calculated, $2 million. They had nothing.

It was 24 September before the group formally registered New Jersey for Hope as a political action committee, launched by money from Young and his colleagues. A month later, the campaign had collected $19,546, much of it in small donations. It wasn't for lack of trying. "We made multiple, multiple calls," says Albowicz.

## On the fence

One obvious ally wasn't biting. The ballot-question backers met twice, hat in hand, with the HealthCare Institute of New Jersey, the trade association for the state's pharmaceutical and device industries. "We really didn't receive anything," Albowicz recalls. Hollie Gilroy, the group's director of communications, says that because of the diverse positions of its 28 member companies, the group couldn't take a stand on the ballot question. "It's up to the individual companies to choose whether or not to support the issue," she says.

As the election drew nearer, the anxiety of the proponents grew. "We had all the plans in the world," says Oster. "But I kept revising the budget down and the overall plan down on a daily basis."

Opponents of the measure had both money and messengers. On 7 October, New Jersey's priests read from their pulpits a written plea from all five of the state's Roman Catholic bishops, asking parishioners to pray that New Jerseyans vote against ballot question two. In a state where 40% of residents are Catholic, that had a large impact. By blanketing every parish, the church, for a "*de minimus*" cost, grabbed the secular media, says Pat Brannigan, the executive director of the New Jersey Catholic Conference.

Newspaper editors weighed in with the fiscal argument. On 11 October, the *Star-Ledger* — the only state-wide newspaper — published a sceptical editorial, suggesting that New Jersey was too debt-ridden to afford another, $450-million loan.

> "Any time you start a campaign where you're under 50%, it's a warning sign."
> — Russ Oster

At Right to Life, Tasy was being quoted regularly in the press, watching the hits on her group's website grow daily and fielding "a lot of phone calls". "I didn't need to hold a press conference," she notes. "The media came to us … They sensed that this was something that could be defeated."

In principle, the advocates for ballot question two had the power of the Democratic legislature behind them — and of Corzine, who had campaigned on a pro-stem-cell platform for his win two years prior. (Corzine and his Commissioner of Health, who was at the time his top stem-cell staffer, declined to be interviewed for this article.) But in practice, most lawmakers were preoccupied with their own re-election races and saw little to gain by campaigning on what was clearly a controversial measure. "We had none of these politicians out there, standing up on this. They all ran for the hills, Democrats and Republicans," says Lonegan.

Lonegan, however, wasn't worried about his lack of high-profile allies. He and his group were assiduously funnelling their arguments to newspaper editorial boards, printing pamphlets referring to the measure as "a half-billion-dollar corporate welfare handout for embryonic stem-cell experiments involving human cloning" and preparing anti-ballot-measure lawn signs.

But Lonegan's greatest impact may have been a television advertisement that he hired Shaftan, the media consultant, to produce. In the ad, which blanketed the state's cable networks for 10 days in October, a charlatan figure hawks "Governor Feelgood's Embryonic Stem-Cell Elixir". "Just $450 million, why that's practically free," he proclaims. "That really pissed them off," says Lonegan.

The news wasn't all dire for Young and his allies. On 22 October, a study was published by Rutgers economist Joseph Seneca and his research associate Will Irving. It had been requested by the legislature, and predicted that the $450-million investment in stem-cell research would return $2.2 billion in economic benefit to the state.

Then on 23 October with shovel in hand before a crowd of photographers, Corzine broke ground in downtown New Brunswick for the Stem Cell Research Institute of New Jersey. Joined by the mother and brother of Christopher Reeve — after whom the building is slated to be named — the governor promised that the facility "will serve as the nexus of cutting-edge scientific breakthroughs that will improve and save the lives of millions of our fellow citizens".

That brief moment was the end of the good public relations, though. From 22 October, the ads featuring McDonald, the paralysed police detective, had been playing all over the state. An Eagleton poll on 25 October showed voter support at 57%; but within a week, Young and his

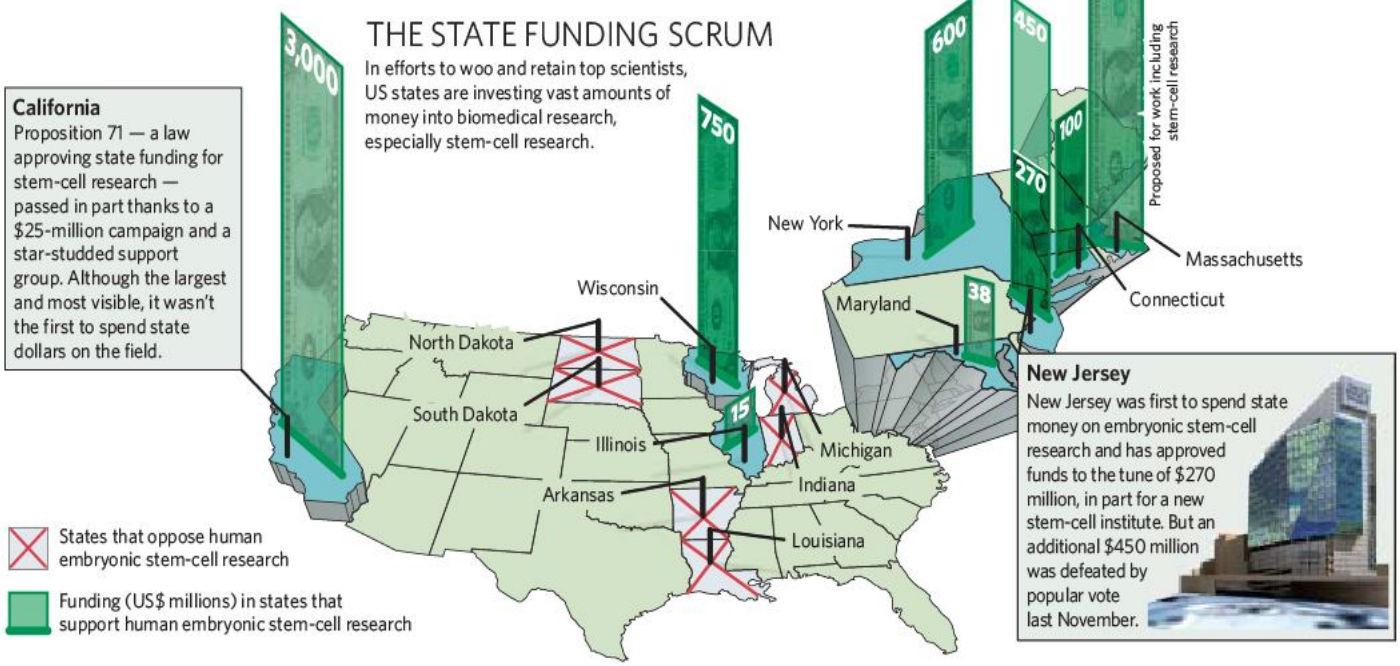
J. BROWN/STAR-LEDGER; M. EVANS/AP

## 26 Oct 2007

Protracted attempts by New Jersey Right to Life and others to remove ballot question two are quashed by the appellate court.

## 06 Nov 2007

Election day: despite a funding blitz at the last minute, proponents of ballot question two mount a meagre campaign and the measure fails 53% to 47%. The next day, Corzine (centre) answers questions about the results.




allies were hearing from unpublished sources that support had slipped to 48%. On 31 October, *The Record*, a newspaper in liberal Bergen County, published an editorial urging voters not to put the state further into debt for a project that the private sector could and should finance. It concluded: "We say yes to stem-cell research, but a loud no to public question no. 2."

Around this time, Albowicz called Young in a panic. "We're going to lose this unless we raise some money now," he said. But with less than $20,000 in the bank, Young says, "We couldn't even get a direct mailing out." They hatched a plan and, together with a fundraiser dispatched from Corzine's office, pooled their phone lists and began making cold calls.

## Cash flow

On 30 October, Corzine sent the campaign $150,000 from his personal bank account. On 2 November, he kicked in another $50,000. That same day, Betsy Johnson, an elderly member of the clan that founded New Brunswick-based Johnson & Johnson added $100,000. Gordon Gund, the Princeton-based former owner of the Cleveland Cavaliers — an Ohio basketball team — sent in $200,000.

> "The politicians ran for the hills, Democrats and Republicans."
> — Steve Lonegan

The infusion allowed the campaign to buy several days of radio ads by Michael J. Fox, ageing rap group the Sugarhill Gang and New York hip-hop artist Styles P. They managed pre-recorded phone calls and one direct mailing to about 400,000 people (The state has 8.7 million.) From 31 October, they reported spending $567,000. They never made it onto television.

On election night, supporters gathered at the West Orange headquarters of Senate president Richard Codey, a key backer of stem-cell research, to watch glumly as the results came in. Early on it was clear that voter turnout had been dismal, even for an off-year election. That was bad news in a battle in which opponents were more motivated to vote than the supporters. The death knell really sounded, however, when it became clear that even the counties of Middlesex and Somerset, where Rutgers and New Brunswick are located, had rejected the measure. In the end, the state's voters defeated the measure by some 79,000 votes, 53% to 47%, voting against it in three-quarters of the state's counties.

Young drew up a post-mortem analysis shortly after the election: turnout in counties that voted against ballot question two was around 32%. Six of those counties opposed the measure by nearly two to one. By contrast, in the five counties that decisively approved the measure — essentially the Philadelphia and New York suburbs — turnout averaged 22% and dipped as low as 10%. Young calculated that if turnout in four of the five pro-stem cell counties had matched the 34% turnout recorded in the 2003 state elections, the measure would have passed easily. The fallout from the loss was immediate. The legislature's Joint Budget Oversight Committee, which had scheduled an 8 November meeting to release the first of the $150 million in construction money, cancelled it the day after the election and has not met since.

A few weeks later, Corzine summoned Young and a handful of other key stem-cell advocates to breakfast at Drumthwacket, the governor's mansion in Princeton. "He said that he would try very hard to ensure that the referendum goes on the ballot again" next November, recalls Young. "But he also explained to us what a difficult situation it was", with the state so deeply in debt.

Stem-cell research supporters in New Jersey clearly missed two key lessons from California: organize big and organize early. California proponents did so out of necessity: under that state's law, they had to gather 600,000 citizen signatures just to get the measure on the ballot. The result: by the time their opposition emerged, the proponents had built a huge, money-and-celebrity-studded machine that gathered more than $25 million, dwarfing the opponents' six-figure fundraising. They also profited from the high voter turnout characteristic in presidential election years.

## Relentless affront

Whether and when another ballot question two will confront New Jersey voters remains unclear. It could be as soon as this November — an idea that has grabbed supporters because of the political advantage that a presidential election is likely to generate. Either way, it won't be long after that, that a new occupant arrives in the White House, with the tantalizing possibility, depending on the winner, of an end to the restraints on federal funding. But in the current flat-funding climate at the National Institutes of Health (NIH), even the most liberal federal policy is unlikely to translate into a big boost. The NIH spent $37 million on human embryonic stem-cell research in 2007. And what funding there is will be the object of fierce competition. All of which leaves Young and his allies determined to press on.

Nevertheless, in New Jersey, which is a liberal state, but also heavily taxed and undeniably pragmatic, a stem-cell friendly victory for the presidency could lead to disinterest in seeing state dollars, already stretched to their limit, being brought to bear on long-term basic biomedical research goals. Composed largely of small towns, small-town politics rule the day in New Jersey, and the lofty dreams of a few politicians and scientists can easily be swept under by more immediate matters such as property taxes, political scandals, and the tolls on the Jersey Turnpike.

Young has steeled himself, however. He and his allies have met several times since the governor's breakfast, hatching plans to turn New Jersey for Hope into a permanent political action committee and lay out a strategy for the next nine months. "I have come to the conclusion that to win next November we don't need $500,000, we need $5 million," Young says. "Dozens" of people, he adds, are hard at work to that end. ■

**Meredith Wadman writes for Nature from Washington DC.**

## Law should recognize value of interspecies embryos

SIR — Considerable time has been spent, during the current debate in the UK Parliament on the Human Fertilisation and Embryology Bill, on the definition and generation of interspecies embryos. A free vote is most likely on this part of the proposed legislation, which is perceived by some to be highly controversial.

We are among those proposing to undertake interspecies somatic-cell nuclear transfer — in which a human cell will be transferred into an animal egg. Our studies will generate embryos in order to advance understanding of genetic reprogramming and nuclear–mitochondrial interaction. They will also generate embryonic stem cells to model certain human diseases. As embryonic stem cells can differentiate into any type of body cell, those harbouring a specific genetic alteration can be differentiated into cells associated with the related disease and studied *in vitro*.

We acknowledge the exciting progress arising from induced pluripotent stem cells, whereby a fully differentiated cell is reprogrammed to behave like an undifferentiated stem cell. However, the success of this process is highly dependent on understanding embryo-derived stem cells. It remains to be determined whether induced pluripotent cells could lead to cancer or other diseases after transplantation, and whether they are equivalent to human embryonic stem cells, as they show different patterns of gene expression[1]. Studying reprogramming through somatic-cell nuclear transfer may improve induced pluripotency and help to produce therapeutically useful cells.

Animal oocytes are a far more reliable source than human oocytes, which are available in very limited supply. Embryonic stem cells have been derived following intraspecies nuclear transfer in mice[2] and monkeys[3], and several cell lines have resulted following transfer of human nuclei into rabbit oocytes[4].

We strongly recommend that scientists be allowed to generate interspecies embryos and to culture these for up to 14 days, placing them under the same restrictions as any human embryos generated under current legislation. We also hope that the new legislation will be revised to allow researchers access to banks of well-characterized tissues and cells that were donated for research but not explicitly for the production of embryonic stem cells by somatic-cell nuclear transfer or other techniques. Such experiments should be conducted in parallel with those generating induced pluripotent cells, to compare the resultant cell lines and to learn more about reprogramming, nuclear–mitochondrial interaction and certain genetic diseases. It would be a retrograde step to prevent any avenue of research, especially one with such high potential gains.

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## Italian neuroscientists are ready to start the debate

SIR — Neurotechnologies such as cognitive enhancement (see *Nature* **450,** 1157–1159; 2007 and *Nature* **451,** 520–521; 2008) and brain reading are likely to have huge social impact. The ethical problems connected with their expected development demand public discussion in our communities. Neuroscientists should take an active part in this debate, as you commented in your Editorial 'Neuroethics needed' (*Nature* **441,** 907; 2006).

We therefore drafted a questionnaire for the 703 members of the Italian Society of Neuroscience (see tinyurl.com/2pwehh). Among other questions, we asked about their interest in neuroethics issues; specific topics discussed in their labs; how they stay up to date; the amount of related reading they do; what specialists they believe are engaged in neuroethics and who they think should be; and how they rate handling of neuroethics issues by the Italian media.

Ten per cent of those surveyed responded. This sample did not vary significantly from the surveyed population with respect to age, discipline or affiliation ($P<0.05$). The answers showed that 91% (95% confidence interval: 84–98) of respondents are interested in neuroethics; 78% (95% confidence interval: 68–88) believe that neuroethics problems should be tackled in collaboration with bioethicists and neuroscientists; and 96% (95% confidence interval: 91–100) would be willing to take part in further initiatives. Neuroscientists under the age of 35 seemed to be the least informed.

Although the small number of responses may indicate a lack of awareness among neuroscientists in Italy about neuroethical issues, the responders show that a significant and representative proportion are interested in public debate.

We offer these results to the community in the hope that our initiative can be replicated on a larger scale. We hope that Italy's media and politicians will exploit the availability of neuroscientists to discuss scientific problems of such outstanding social interest.

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## Darwin's legacy makes its mark in Croatia

SIR — Charles Darwin's *On the Origin of Species* and *The Descent of Man* have at last been translated into Croatian, thanks to the work of the renowned science and theology translator Josip Balabanić. Other European countries — including Denmark, the Netherlands, France, Germany, Italy, Poland, Russia and Sweden — had access to Darwin's works in their mother tongue during his lifetime. But it was not until this year that Croatian students of biology could read them in their own language.

Religious education was introduced in elementary schools during the early years of Croatian independence, and ethics and the major world religions are now studied in high school. At the same time, the importance of evolution for modern biology and medicine is publicly acknowledged by science academies and societies — in the spirit of your Editorial 'Spread the word' (*Nature* **451,** 108; 2008).

Croatia aspires to join the group of countries in which education and science occupy prime positions in national strategies, and recognizing the influence of Darwin's writings is an important step in that direction. Celebrations of the 200th anniversary of Darwin's birth on 12 February next year, possibly at the Croatian Academy of Sciences and Arts, will have particular significance for Croatians.

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# BOOKS & ARTS

'Quack' John R. Brinkley and his wife at their Kansas clinic.

KANSAS STATE HIST. SOC.

# *JAMA* and the mountebank

**A timely tale of one man's mission to stamp out medical fraudsters in 1920s America.**

**Charlatan: America's Most Dangerous Huckster, the Man Who Pursued Him and the Age of Flimflam**
by Pope Brock
Crown Publishers: 2008. 304 pp. $24.95

**Michael Shermer**

Human cognition has a problem — anecdotal thinking comes naturally whereas scientific thinking does not. The recent medical controversy over whether vaccinations cause autism illustrates this barrier. On the one side are scientists who have been unable to find any causal link between the symptoms of autism and the vaccine's ingredients. On the other are parents who noticed that shortly after having their children vaccinated autistic symptoms appeared.

Anecdotal associations are so powerful that they cause people to ignore contrary evidence. In the vaccination case, the imagined culprit for autism's cause is the preservative thimerosal. Yet it breaks down into ethylmercury, which is expelled from the body too quickly to have a damaging effect (plus autism continues to be diagnosed in children born after thimerosal was removed from vaccines). The story holds power despite the contrary facts.

The reason for our cognitive disconnect is that the brain evolved to be cautious. We favour anecdotes because false positives (believing there is a connection between A and B when there is not) are usually harmless, whereas false negatives (believing there is no connection between A and B when there is) may take you out of the gene pool. Our brains are 'belief engines' that seek connections.

This faith in anecdotes can make us easy to exploit. Any medical huckster promising that A will cure B has only to advertise a handful of successful testimonials. Enter John R. Brinkley, one of the most notorious medical quacks of the first half of the twentieth century, and his nemesis Morris Fishbein, the quackbusting editor of the *Journal of the American Medical Association* (*JAMA*). Their long struggle throughout the 1920s and 1930s, wonderfully retold in a gripping narrative by Pope Brock, brings to life this tension between folk and scientific medicine.

As Brock ably demonstrates, Brinkley came of age on the tail end of the freewheeling 'patent remedy' era in which con-men hawked their folk medicine out of the side of wagons:

> "They usually performed at night. A platform was unfolded and torches placed at each corner as the audience gathered, drawn by handbills and word of mouth. First a fiddler or a dancer got the crowd warmed up. A short morality play followed, in which a noble head-of-house or ringleted female died pathetically for lack of a miracle tonic, identified by name. Finally the physician himself (Brinkley) shot onstage in a dinner-plate hat, cutaway coat and pious pants that buttoned up the sides, theeing and thousing, singing and selling, waving a bottle of Ayer's Cathartic Pills. Or maybe Burdock Blood Bitters or Aunt Fanny's Worm Candy. One thing was for sure, whatever it was cured whatever you had."

What many men had, Brinkley discovered as he honed his scam, was a lack of sexual vitality. He developed a surgical technique that offered the type of firm results that his male clientele so desperately sought: goat testis sewn into the patient's scrotum, which he likened to "embedding a marble in an apple". Come one, come all. And they did, to the tune of $750 per surgery, advertised widely in newspapers (more than half of all newspaper advertising at the time was for patent remedies) and on radio, the

new-fangled technology that Brinkley took to like an evangelist to television.

It made him a rich man. But as his business grew he got careless, performing operations both before and after happy hour, and fobbing off work to assistants whose medical credentials were even shadier than his own (Brinkley graduated from the improbably named and then unaccredited Eclectic Medical University of Kansas City). "Dozens of patients died over the years, either in the operating room or shortly after their return home," Brock explains. "Many others were permanently maimed."

This attracted the attention of the ambitious medical writer Morris Fishbein, whose career coincided with an attempt by the American Medical Association (AMA) to stamp out charlatanism through accrediting medical colleges and licensing practitioners. Fishbein made his public mark in 1923 when the *Chicago Daily News* sent him to investigate the 'Hot Girl of Escanaba', a woman who suffered from a temperature of 46 °C for two weeks. Fishbein exposed her as a "hysterical malingerer" when he found a flesh-coloured hot-water bottle was used to elevate rectal thermometer readings.

"Along with making him famous as a fraud-buster extraordinaire," Brock notes, "the case fixed him in a role he would revel in for years to come: the face, the popularizer, the lord high priest of the AMA." For the next two decades Fishbein pursued the country's "most daring and dangerous" swindler, as he called Brinkley, until he finally brought him down in a decisive court room confrontation that reads like a Hollywood film script.

Stripped of his licence to practise medicine and embroiled in lawsuits, Brinkley moved to Mexico where he dispensed pseudo-medical twaddle over the airways through a 'border-blasting' radio station that could be heard all the way to Canada. When the Mexican government shut him down in 1941 — in part because of his public sympathies for the Nazis — he was a broken man. "My health is gone. I am ready for the bed and out," he wrote to his wife three days before a heart attack killed him, aged 56.

Fishbein's promotion of science-based medicine was heroic in his day. More than half a century later, medical flimflammery still flourishes on the Internet. Every medical association and journal needs a quackbusting Fishbein on its staff, for without such eternal vigilance, folk medicine will trump scientific medicine in the minds of patients. ■

Michael Shermer is adjunct professor of economics at Claremont Graduate University, Claremont, California 91711, USA. He is publisher of *Skeptic* magazine (www.skeptic.com) and author of *The Mind of the Market*.

# Multi-dimensional lives

**Finding Moonshine: A Mathematician's Journey Through Symmetry**
by Marcus du Sautoy
Fourth Estate/Harper: 2008.
400 pp/384 pp. £18.99/$25.95

**Mark Ronan**

The mathematics of symmetry emerged from the work of Evariste Galois, the young genius who died aged just 20 in 1832 after being shot in a duel. His tragic story, placed in the context of French history and revolution, is retold here in Marcus du Sautoy's follow-up to *The Music of the Primes*. Galois's is a dramatic story of strong egos and lost manuscripts, ending with a letter he wrote the night before he died — perhaps the most famous epistle written by a mathematician.

Galois was working on the solution of equations, a problem first tackled some 4,000 years earlier when the Babylonians created a formula for solving quadratic equations. The subsequent theory was developed by several main characters: the twelfth-century Persian mathematician, astronomer and poet Omar Khayyam; four Italians in the early 1500s; Paolo Ruffini in 1799; and Niels Henrik Abel in 1824, the young Norwegian who showed that most fifth-degree equations and higher cannot be solved in terms of square roots, cube roots and so on.

Some equations of high degree could be solved in this way, but a method was needed to determine which ones they were. Enter Galois. By examining patterns among the solutions and studying the group of symmetries preserving these patterns, he could solve the problem. If the group of symmetries could be deconstructed into cyclic groups, then the solutions could be expressed in terms of roots. Some groups do not admit any deconstruction — they are 'atoms of symmetry' — and Galois found the first ones.

Atoms of symmetry are the basic building-blocks for all finite groups of symmetry, and some of them have applications in modern technology. For example, they can be used to encode digital data such that small transmission errors can be automatically and efficiently corrected. Most symmetry atoms fit into a 'periodic table' where they belong in one of several families whose members enjoy similar properties. There are 26 exceptions. The largest of these is the 'Monster', a vast group of symmetries requiring at least 196,883 dimensions in which to operate. It exhibits numerical patterns similar to those obtained in an important branch of number theory, a connection dubbed 'Moonshine' by John Conway, who was one of the first to investigate it and marvel at its surprising magic.

Islamic tile patterns display a multitude of symmetries.

PRIVATE COLLECTION/STAPLETON COLLECTION/BRIDGEMAN ART LIBRARY

Moonshine appears at the end of *Finding Moonshine*; the main thrust is elsewhere. Twelve chapters, one for each month of the year, include descriptions of the author's own life and work in the months concerned. Du Sautoy describes how he conducts his own research, interacts with other mathematicians, his family and particularly his son. He points out that mathematicians can be strange people, and pokes playful fun at the idiosyncracies of some of those who worked on the Monster. He is admirably self-deprecating, recounting how on a visit to Japan he annoyed local guests by remarking that the sake was only 30° proof, which is not a prime number, whereupon his host obligingly produced a 43°-proof alternative.

Interesting interludes highlight the elementary aspects of symmetry, including its role in the music of Bach. Du Sautoy also discusses the regular solids (tetrahedron, cube, and so on), teaching us that the Romans were so obsessed with dice games that they carried heavy gaming boards on campaigns, and even used 12-sided dice invented by the Etruscans. He says that Pythagoras learned of the dodecahedron from the Romans while in southern Italy. But hold on — when Pythagoras moved to Croton in Italy in the sixth century BC, Rome was an Etruscan kingdom. The Romans came later. Inaccuracies such as these, the lack of references and the projection of unverified feelings onto historical characters, spoil an otherwise delightful account of the early history (particularly of the sixteenth-century Italians) and the subsequent research on equations. Later material leading to the Monster also contains some factual errors.

The appearance of the Monster near the end is where the mathematics gets most interesting and relevant to more applied areas of science. For example, symmetry atoms are used in physics to create the standard model of the quantum forces, and Moonshine finds a home in string theory.

That said, du Sautoy omits the applications to physics, and sticks with simple symmetries that a reader with no mathematical appreciation will understand. I, meanwhile, share Freeman Dyson's sneaking hope that "some time in the twenty-first century, physicists will stumble upon the Monster group, built in some unsuspected way into the structure of the Universe". ■

Mark Ronan is professor of mathematics at the University of Illinois at Chicago, Illinois 60607, USA, and honorary professor at University College London. He is author of *Symmetry and the Monster*.

EXHIBITION

# Capturing colours of times past

Laura Spinney

In these days of digital cameras and Photoshop, it is hard to imagine the wonder that instant photography and the first colour photographs inspired. It comes as a revelation to witness a landscape striped by lightning in daylight through the eyes of the photographer who first captured it; to stand beside him in a mocking crowd, as he points a camera at fireworks exploding in the night sky; or to feel his embarrassment as he asks the King and Queen of Denmark to hold their poses for a few seconds.

In all three cases, the photographer was the Frenchman Léon Gimpel. He took up photography just as it was moving away from science to become an art, but was still developing rapidly. His inventive use of the medium helped drive that development. Today Gimpel is barely known because the techniques he experimented with and improved upon were superseded and became obsolete. In his lifetime, his work was considered revolutionary.

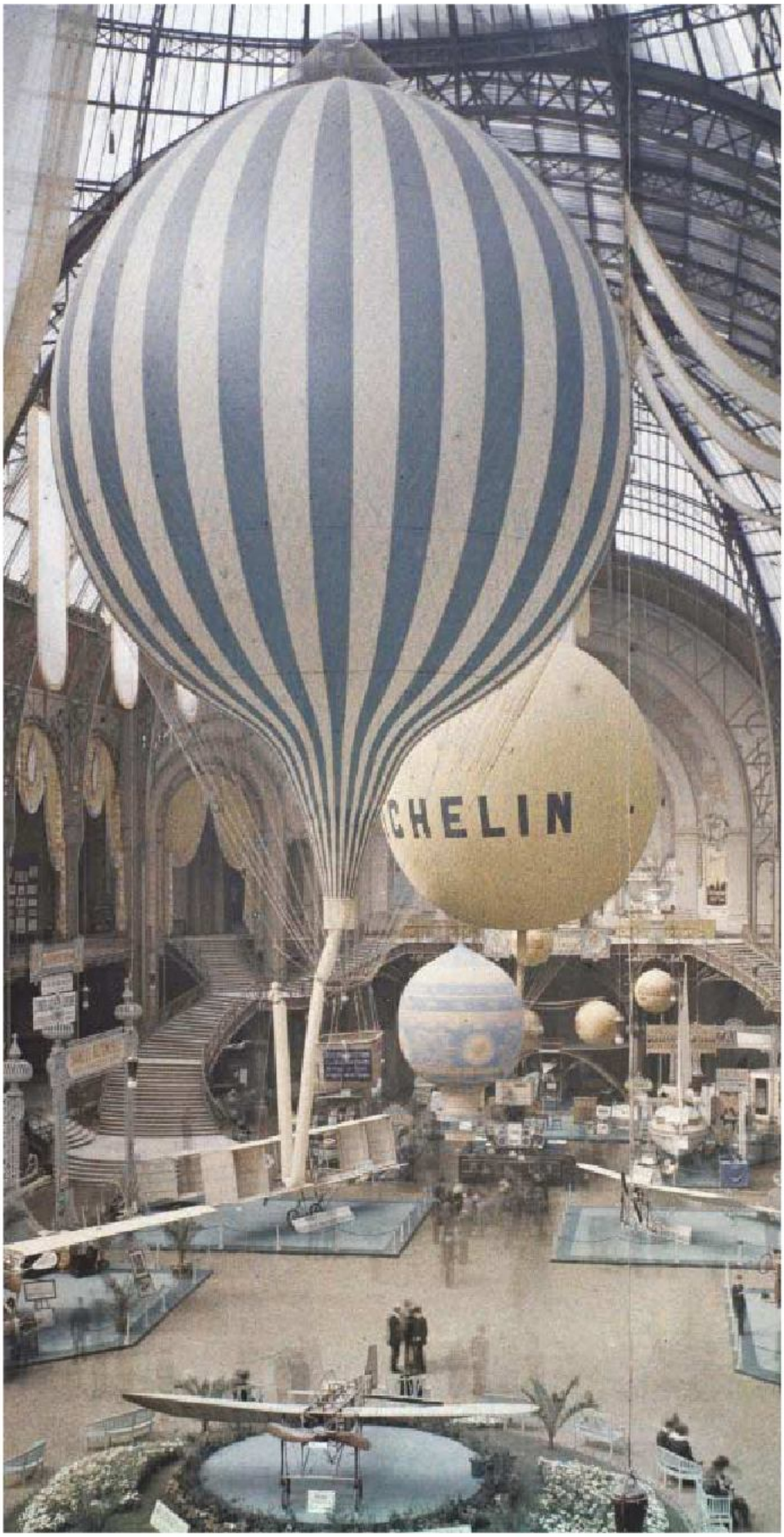
Gimpel's images are the subject of a new exhibition at the Musée d'Orsay in Paris, organized in collaboration with the French Society of Photography. The pictures are accompanied by extracts from *L'Illustration*, the newspaper to which he contributed for 30 years.

Using autochrome, a predecessor of colour photography using starch on a glass plate patented by the Lumière brothers in 1907, Gimpel was one of the few to capture the colours of the Belle Epoque — the 'beautiful era' from the 1890s to the start of the First World War. With others he found a way of increasing the sensitivity of the autochrome plate, so that he no longer had to use a tripod or ask his subjects to sit patiently. Thus colour photography became instant. Gimpel exploited the innovation to chronicle the technological advances of the age, from the birth of aviation to the advent of neon light. He also captured human scenes — the nocturnal life of Paris; the poorly lit interiors of pre-war France.

Each image is also a tale of technical obstacles overcome, of curiosity, experimentation, perseverance and triumph. Many appeared first in the pages of *L'Illustration*, where their reproduction was itself a feat, and mounting the fragile glass autochromes has in turn tested the exhibition's curators. Backlit by a cold light, what is so striking about the colours is how pale and delicate, not to say washed out, they are to over-stimulated, modern eyes, and somehow, all the more moving for it. ■

Laura Spinney is a science writer based in London and Paris. Her latest novel, *The Quick*, is out in March.

***Léon Gimpel*** **runs from 12 February to 27 April at the Musée d'Orsay, Paris (www.musee-orsay.fr).**




COLLECTION SFP

Gimpel's autochrome of the first 'Exposition Internationale de Locomotion Aérienne' in Paris, 1909.

IN RETROSPECT

# Diagnosing deep similarity in nature

**On the Nature of Limbs: A Discourse**
by Richard Owen
University of Chicago Press: 2007. 119 pp.
$50 (hbk), $20 (pbk)

**Michael Coates**

On the night of 9 February 1849, Richard Owen, the pre-eminent Victorian anatomist who later founded London's Natural History Museum, delivered a public lecture at the Royal Institution of Great Britain. In a strident discourse that set the stage for Charles Darwin's account of evolution, Owen revealed similarities in biological forms from species to species that suggested some underlying ideal plan or archetype. He sought to debunk the prevailing view that anatomical pattern could be explained as a consequence of biological function. With example after example, Owen hammered home the point that structural correspondences between species, or even between parts of the same individual, cannot be explained simply by adaptation. The echoes of his words still reverberate.

Owen's ideas contributed directly to Darwin's synthesis of evidence for evolution a decade later. Where Owen saw an archetype — an abstracted common pattern — Darwin glimpsed an ancestor or ancestral condition. "Nothing," Darwin wrote in *On the Origin of Species*, "can be more hopeless than to attempt to explain this similarity of pattern in members of the same class, by utility or by the doctrine of final causes."

My first reaction to re-reading Owen's *tour de force* was that it must have been a slow evening. In long-winded prose, Owen pores over the minutiae of vertebrate limb bones, the mistakes of lesser scientists and the relationship between observed anatomy and his theoretical template for vertebrate-kind. Many readers might be tempted to skip the text and turn to the pictures, especially the classic plate depicting Owen's own vertebrate archetype, like a skeletal zeppelin hovering above human, fish, bird and other osteological comparisons. Incidentally, the figure caption is vast, and almost qualifies as further discourse on the themes in the main text. Yet it is worth persisting. Brian Hall's preface and introductory essays by Mary P. Winsor, Jennifer Coggon, Ron Amundsen and Kevin Padian bring out the subtleties of Owen's arguments and place them in a historical and social context, among the philosophical turmoil of biology in the early to mid-nineteenth century.

A decade before Darwin's *On the Origin of Species*, Owen very nearly sketched a theory of evolutionary transformation, fragments of which appear here. However, as Padian describes, such were the sociopolitical and philosophical strains on Owen's position that he stalled at the final intellectual leap. Owen's patrons were of the Oxbridge-educated establishment — adherents to the natural theology of the 'argument from design' (for the existence of God) as advocated most influentially by William Paley (now sadly repackaged with a molecular gloss by the proponents of 'intelligent design').

Uncomfortably for Owen, his explanations of form raised the loathsome prospect of atheism. They drew heavily on the ideas of European philosophers and anatomists, such as Lorenz Oken and Carl Gustav Carus, bringing British comparative biology up to speed with that on the continent. That these notions required no beneficent deity, which created perfect organisms with optimally designed body parts, placed the ambitious Owen in a professionally risky position. Consequently, *On the Nature of Limbs* includes some curiously religious passages about the quest for the archetype, equating it with the discovery of the 'divine plan' (an aspect of Victorian academia of interest, perhaps, to readers of Philip Pullman's *His Dark Materials* trilogy).

As an account of the details of vertebrate limbs and fins, *On the Nature of Limbs* could be filed away as of historical interest only. But it remains an excellent source for those interested in how we identify and interpret pattern in nature. A dissertation on similarity, conservation and variability in form, it addresses issues of enduring interest to systematic biologists as well as to the revitalized field of evolutionary developmental biology. The book is a superb vehicle for the exploration of homology, Owen's most lasting legacy to modern biology. Homology is the diagnosis of deep similarity in nature, based upon simple criteria — position, development and structure. Manifestations of homology have been explained variously in terms of archetypes and evolution, separately and in combination, and a reading of Owen's discourse offers a refreshed perspective on this sometimes slippery concept.

Ron Amundsen emphasizes Owen's stand for a structuralist perspective in biology as being an essential step on the road to current understanding that adult form and embryonic development are contingent upon evolutionary history. Even so, Owen remained resolutely in the camp of non-evolutionary biologists. For Owen, just as fins and limbs were variations on the extremities of an ideal vertebra, so actual species could be thought of as departures "from the first embodiment of the Vertebrate Idea under its old Ichthyic vestment, until it became arrayed in the glorious garb of the Human form".

A contentious character, Owen is all too easily dismissed as the self-promoting, vitriolic critic of Darwin. *On the Nature of Limbs* is a timely reminder that his work was — is — of real value. ■

Michael Coates is associate professor in the department of organismal biology and anatomy at the University of Chicago, Chicago, Illinois 60637, USA. He is a co-editor of *Evolution & Development*.

FASHION

## Fleeting fabrics

Imagine if last season's dress or suit vanished of its own accord after some expiration date. In a comment on our disposable culture, Valentino-trained fashion designer Helen Storey is using know-how from materials science to make a show of frocks that dissolve slowly in water.

Her couture creations, made from biodegradable polymer threads, are being publicly drowned in a gallery window near London's busy shopping hub, Oxford Street. Storey has long harboured concerns about our attitudes to waste and recycling, and during her career has woven plastic refuse bags and reused rags to make boas and evening gowns.

To realize her idea of evanescent products, such as packaging that disappears as its contents expire, Storey contacted chemist Tony Ryan, of the University of Sheffield, UK, after hearing him on the radio. Their *Wonderland* collaboration has produced new textiles and several patented products, including a water-purification device, a biodegradable bottle and orthopaedic shoes.

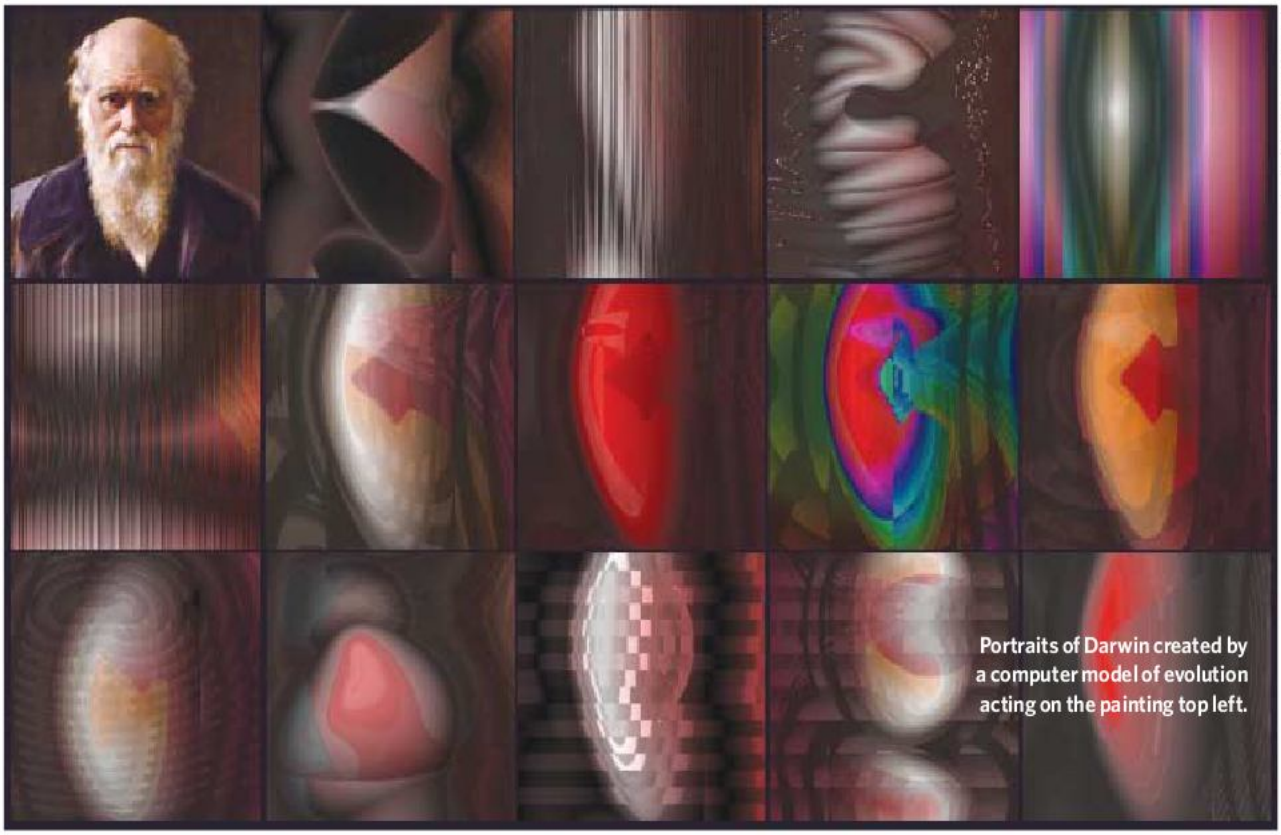
A. MAGUIRE

Ryan relishes the injection of wild ideas into his lab: "Helen really pushes the boundaries of our knowledge, but we share the dream of a world that uses its resources more responsibly." J.B.

***Wonderland* is showing at London College of Fashion (29 January to 29 February), moving to Sheffield and Belfast later in the year. See www.showstudio.com/wonderland.**

# Darwin's enduring legacy

As the 200th year since the great naturalist's birth begins, **Kevin Padian** looks forward to a season of celebration by outlining how Darwin's ideas changed scientific thinking.



S. DIPAOLA/IVIZLAB.SFU.CA

Portraits of Darwin created by a computer model of evolution acting on the painting top left.

**Kevin Padian**

Perhaps no individual has had such a sweeping influence on so many facets of social and intellectual life as Charles Darwin, born on 12 February 1809. Of the other two of the great nineteenth-century triumvirate of European thinkers, Marx's ideas have been distorted beyond recognition in their political execution, and Freud's approach to the psyche no longer merits scientific recognition. Neither man had Darwin's impact on the structure of empirical knowledge.

In the past century and a half, Darwin's ideas have inspired powerful images and insights in science, humanities and the arts. Meanwhile, countless commentators ignorant of his meaning have borrowed his eloquence to plump their own chickens — from capitalism to 'evolutionary psychology'. Darwin has been invoked as the demon responsible for a variety of perceived heartless ills of society, including atheism, Nazism, communism, abortion, homosexuality, stem-cell research, same-sex marriage, and the abridgement of all our natural freedoms. One can scarcely imagine the horror that Darwin would feel at the misunderstanding, misappropriation and vilification of his ideas in the 125 years since his death.

As we prepare to mark next year the 200th anniversary of Darwin's birth and the 150th of the publication of *On the Origin of Species*, it is an opportune time to reflect on just what constitutes Darwin's enduring greatness in Western thought. His contributions can scarcely be reduced to a simple list, but the following ten topics hint at the magnitude of the man's legacy.

## Grandeur in this view

**Natural selection** Both Darwin and his co-discoverer of natural selection, Alfred Russel Wallace, were partly inspired by the social economic theory of Thomas Malthus. Malthus noted in his great *Essay on the Principle of Population* (1803) that population growth would always outstrip resource growth, so overpopulation and insufficient supply are inevitable and should be accepted and dealt with. Darwin and Wallace independently applied these principles to the natural world. More offspring are produced than can survive; some are better suited to the prevailing conditions than others; and those better-suited individuals are more likely to leave their advantageous heritable features to the next generation. Malthus may have been the godfather of the workhouses, designed to deter citizens from insolvency and dependence on the public weal, but his bleak view of amelioration was not Darwin's, any more than was Herbert Spencer's appropriation of natural selection for his social manifesto of the "survival of the fittest".

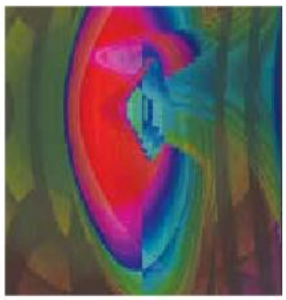
Darwin was less emphatic than Wallace about the pre-eminence of natural selection among other mechanisms of evolutionary change. But he did think it was important, and it provided a plausible process for the transmutation of species that made the concept of common descent of all species respectable, given what was understood of heredity in Darwin's day.

On the other hand, mathematicians ignorant (like Darwin) of the genetic underpinnings of heredity soon produced demonstrations that natural selection could have little real effect on species, and the whole idea fell into some disfavour, even in Darwin's lifetime. It was rescued, ironically, by the mathematical modellers of the Modern Synthesis of evolutionary theory in the 1930s. Ronald Fisher, Sewall Wright and J. B. S. Haldane showed, among other things, that even small selective advantages could permanently affect evolution in populations. They brought back natural selection with a quantifiable vengeance, and it has been

the primary focus of evolutionary research ever since.

**One tree of life** A sketch Darwin made soon after returning from his voyage on *HMS Beagle* (1831–36) showed his thinking about the diversification of species from a single stock (see Figure, overleaf). This branching, extended by the concept of common descent, eventually formed an entire 'tree of life', developed enthusiastically by his German disciple Ernst Haeckel in the decades following the *Origin*. The unity of life gained independent confirmation, of course, with the discovery of genetic structure more than a century after the *Origin* was published.

**Genealogical classification** Before the acceptance of common ancestry, classifications were attempts to discern some shadowy philosophical or theological 'natural system' to organize biological similarity. The tree of life implied that relatedness was what Darwin called the "hidden bond underlying all our classifications". He insisted in letters and books throughout his career that classifications should be, as far as possible, genealogical. Yet in his own work on barnacles he found it difficult to construct classifications based solely on common ancestry, because the animals had been so highly modified. This led to another essential, often overlooked, keystone of his work:



**Selective extinction** Extinction had been recognized as a fact since the mid-eighteenth century. It took Darwin to recognize the extent to which it has shaped the contours and lacunae of diversity through time. Far from preserving a "great chain of being", the living world is a patchwork of possible forms, with most transitional stages and features removed. This explains at once why it is so easy to separate living things into discrete major groups (phyla and so on) and why it is so difficult sometimes to link them, as Darwin found with his barnacles.

**Deep Time** This apt phrase was not known in Darwin's time, but was an inevitable concept. Darwin wrote in the *Origin* that any reader who has not grasped the incredible stretch of time required for biological evolution "may at once close this book". He was serious. He calculated the period necessary to build up and wear down many of the major rock formations of England, to underscore the point. True, Lord Kelvin's calculated limits on solar duration nonplussed many supporters of Deep Time, but Darwin was not cowed by physics, because he knew the rocks. Deep Time was absolutely necessary to his theory, in a way that it had not been for any biological theory before. It was no longer possible to accept that Earth was 6,000 years old, as some Biblical scholars estimated.

**Biogeographical distributions** If species can diversify, if they change by adapting to new circumstances and opportunities, if they can migrate, and if climates have changed through time, then the distributions of plants and animals are not serendipitous patterns or whims of a Creator. Darwin saw that upland and lowland rodents in North and South America were most closely related to their continental neighbours, not to their ecological counterparts on separate continents. Only evolutionary adaptation and dispersal could account for such patterns.

In Darwin's day, dispersal through migration was the only mechanism thought possible for species to move among continents. A century after the *Origin*, plate tectonics provided the second major mechanism for moving species and changing biogeographic distributions.

**Sexual selection** Darwin realized that other forces besides natural selection might influence the evolution of form in species. He noticed that the differences between sexes in many species, from beetles to marine invertebrates to birds and mammals, could be important in mate choice, and that this would affect the reproductive success of the next generation. His exposition of sexual selection was the subtitle of the otherwise curiously named *The Descent of Man*. Differences between sexes could now be explained as the result of processes of mate choice and territorial competition, not merely as of divine design.

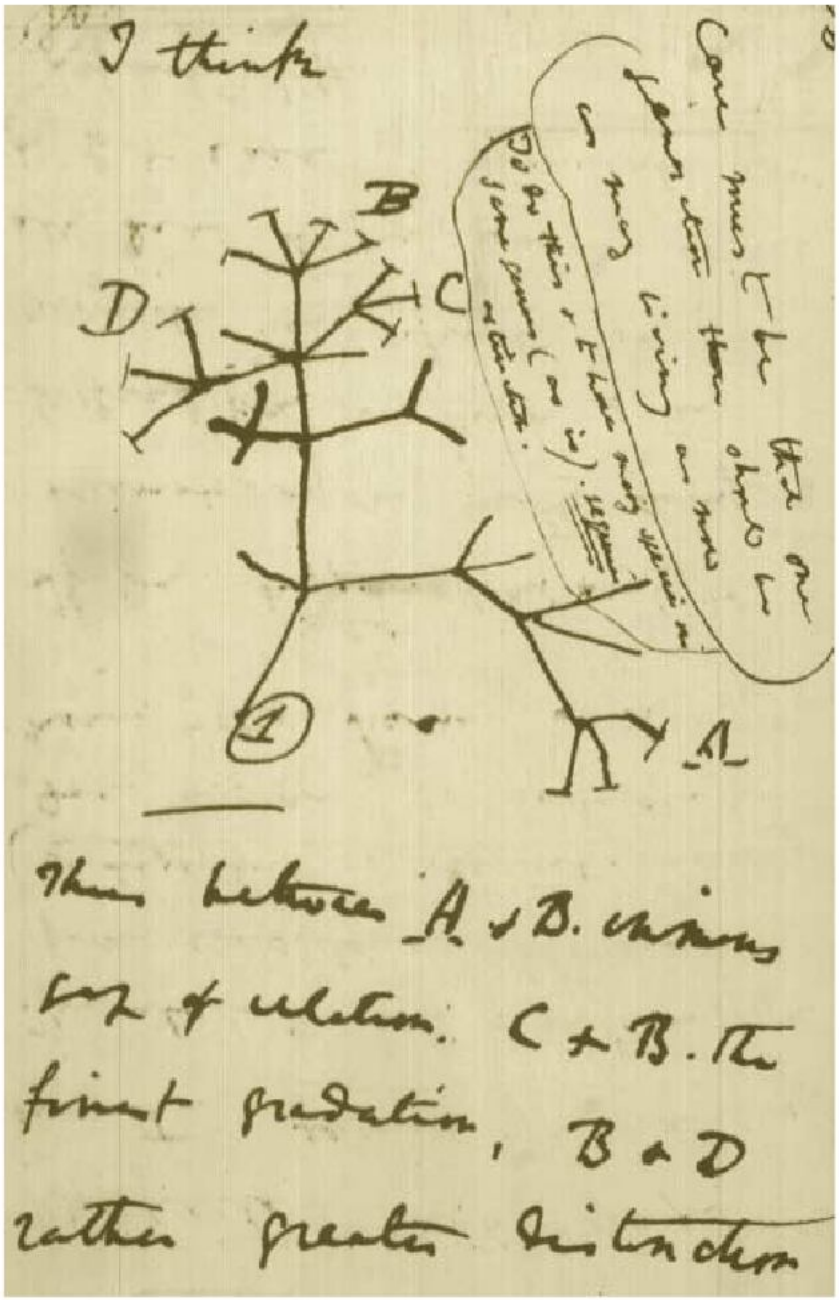
**Coevolution** One of Darwin's lesser-known books is *On the Various Contrivances by which British and Foreign Orchids are Fertilised by Insects and on the Good Effects of Intercrossing* (1862). It encapsulates the concept that species of very different origins have evolved mutual ecological relations through time that have come to affect critical aspects of their morphologies. An African orchid was discovered that had a corolla nearly a foot long. Darwin inferred that there must be a moth with a tongue long enough to extract its pollen. When the moth sub-species was eventually discovered, it was given the name *praedicta*. Today we can identify groups of plants and their insect predators, vertebrates and their parasites, lichens composed of an alga and a fungus, and many other associations that can only reasonably be explained by co-evolution through diversification over millions of years.

**Economy of nature** Darwin invented many ideas that currently constitute the science of ecology, although the word 'ecology' was unknown in his day. He developed the idea of "the economy of nature" — the inter-relations of species in a community, like businesses and consumers in a society. What had been, for earlier authors, the divinely ordained balance of nature became the autocatalytic war of nature. He acknowledged the "tangled bank" of species in a community, and outlined the dynamic between biological and physical influences on the distribution and survival of species. Darwin became increasingly persuaded of the importance of biological processes, and his emphasis on the influences of competition and predation on survival continues to dominate ecology.

**Gradual change** It is widely, and correctly, recognized that Darwin promoted gradual change. But what did he mean by 'gradual'? Most dictionary definitions have it as 'slow and steady', and this is indeed one meaning that Darwin used. There is another. On the *Beagle* voyage, Darwin landed at Concepción, Chile, just after a great earthquake had demolished hundreds of buildings, killed and injured many, caused a huge tsunami and thrown the cliffs several metres higher up along the coastline, leaving putrefying sea creatures still attached to the formerly submerged rocks. Darwin, inspecting the coastline the next day, and seeing evidence of many such changes in hundreds of feet along the terraced cliffs, described this in his journal as a "gradual" change. To understand why, we must consider the etymology of the word 'gradual', which comes from the Latin *gradus*, meaning 'step'. The geological change was step-like: gradual. This conceptual tension between 'slow and steady' and 'step-like' is the basis of one of the most important evolutionary ideas of the twentieth century: punctuated equilibria. This generalization is based on myriad fossil examples showing that the morphology of species may not change appreciably for much or most of their history, then alter relatively rapidly. If this turns out to be the predominant pattern of evolution in well-preserved fossil sequences, as now seems to be the case, Darwin's view of the plurality of evolutionary tempos and modes will be vindicated.

## From so simple a beginning

The list above, which is by no means complete, prompts the question: has any single individual made so many lasting contributions to a broad area of science as Darwin




CAMBRIDGE UNIV. LIB./DARWIN-ONLINE.ORG.UK

**Charles Darwin's 1837 sketch of the diversification of species from a single stock.**

did to biology? Certainly the history of Western thought is graced by many polymaths and geniuses and comparisons are ultimately meaningless.

But Darwin moved intellectual thought from a paradigm of untestable wonder at special creation to an ability to examine the workings of that natural world, however ultimately formed, in terms of natural mechanisms and historical patterns. He rooted the classification of species within a single branching tree, and so gave systematics a biological, rather than purely philosophical, rationale. He framed most of the important questions that still define our understanding of evolution, from natural selection to sexual selection, and founded the main principles of the sciences of biogeography and ecology. His work is still actively read and discussed today, inspiring new students and scientists all over the world. Few authors can claim so much.

It is dismaying, then, to note the rise of anti-evolutionism in recent decades. This is a direct result of the rise of religious fundamentalism, whose proponents feel it necessary to reject modern science on the basis of highly questionable (from mainstream historical and theological viewpoints) readings of sacred texts.

## Divergence of character

One might well ask how such people can accept the benefits of medical research, inoculations, pharmacology, crop improvement and so much more that depends on an understanding of evolution. Most of them reject the evolutionary basis of these advances, regarding it simply as 'variation' that can be selected like features of dog breeds. This is why 'microevolution' in populations poses little threat to fundamentalists, and perhaps why even most evolutionary scientists (dominated by population biologists) have not been intensely engaged in the defence of evolution against its detractors. There is no evidence that Darwin thought in modern populational terms, but he did feel that the changes in species wrought by natural selection and other processes would eventually lead to new kinds of organisms with new adaptations — a premise violently rejected by fundamentalists and other anti-evolutionists.

Happily, in one non-scientific arena at least, an honest, almost organic understanding and appreciation of Darwin has flourished. This is in literature, where authors from George Eliot to John Fowles have consciously or unconsciously absorbed his precepts and insights, nourishing beautiful prose and poetry. None, perhaps, more so than Thomas Hardy, who intuitively understood Darwin's layers of deep time, historical contingency, hereditary predilections and weaknesses, environmental opportunities, the various scales of change that comprise evolution, the constant need to adjust — and especially the insignificance of individuals against the great flow of life and time.

As Hardy put it: "Let me enjoy the earth no less / Because the all-enacting Might / That fashioned forth its loveliness / Had other aims than my delight." This child of the Enlightenment was well aware of more ancient world views, and humbled by what the new investigations of the cosmos revealed. Humans are animals, one species of many on the planet, bound by common ancestry to all other species, part of an ages-old dance of reproduction, accommodation, survival and alteration.

It is for this vision, one that liberates humans from the conceit of special creation, that Darwin was honoured by interment in Westminster Abbey. And it is for his innumerable scientific insights, most still as valid and stimulating as the day he coined them, that we look forward to celebrating him next year. ■

Kevin Padian is professor of integrative biology and curator in the Museum of Paleontology, University of California, Berkeley, and president of the National Center for Science Education, Oakland, California. He served as an expert witness in the Dover, Pennsylvania, 'intelligent design' trial in 2005.

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nature REVIEWS MOLECULAR CELL BIOLOGY

# Free Poster – Targeting lipid signalling in disease


Lipids are important mediators in cancer, inflammation and in cardiovascular, neurodegenerative and metabolic diseases. A complex protein–lipid signalling network that comprises phosphoinositides, sphingolipids, steroids and other lipid-derived mediators has been uncovered in recent years.

This Poster provides a schematic overview of the protein–lipid signalling network and how this network can be exploited pharmacologically to attenuate proliferative, inflammatory and metabolic diseases.

The Poster accompanies a special Focus on Lipids (www.nature.com/nrm/focus/lipids), the content of which is freely available throughout February 2008.

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NEWS & VIEWS

EVOLUTIONARY BIOLOGY

# Ancient bacteria liked it hot

Manolo Gouy and Marc Chaussidon

**Proteins from ancestral bacteria have been modelled and reconstructed. Strikingly, the heat stability of these proteins parallels the temperatures of their ocean habitats, as determined from the geological record.**

The study of the environment in which early life evolved has long been the domain of the physical sciences. For example, analyses of the chemical and isotopic compositions of rocks formed during the Archaean (from 3,800 million years ago to 2,500 million years ago) allow precise dating and reconstruction of environmental parameters at that time, such as seawater temperature and atmospheric composition. But the natural sciences have recently discovered other ways to study the habitats of early life. On page 704 of this issue, Gaucher *et al.*[1] describe one such approach. They have 'resurrected' proteins from bacteria of bygone ages, providing clues about the temperatures at which these organisms lived.

The protein targeted by Gaucher *et al.*[1] is elongation factor thermo-unstable (EF-Tu), a crucial player in protein synthesis. Every extant cell contains variants of the genes that encode EF-Tu, and so the factor is expected to have existed in all cells throughout evolutionary history. In present-day bacteria, the temperature above which EF-Tu loses its precise three-dimensional structure is highly correlated to the temperature of each species' habitat — EF-Tu proteins of hyperthermophilic bacteria (which thrive at temperatures of 80 °C or more) maintain their structures at high temperatures, whereas the analogous proteins of mesophilic bacteria (which prefer temperatures between 25 °C and 40 °C) unfold at much lower temperatures.

The sequences of many extant bacterial genomes have been determined, so EF-Tu sequences from species representing various groups and habitats are known. By contrast, ancient protein sequences can only be estimated, using methods based on statistical modelling of the molecular evolutionary process. Gaucher *et al.*[1] have made such estimations for a dozen organisms in the bacterial phylogenetic tree, starting from the last common ancestor and ending with present-day bacteria. They synthesized the corresponding gene sequences and cloned them into *Escherichia coli* cells, which then produced ancestral EF-Tu proteins. The authors then measured the thermostability of the proteins.

The results are striking. In bacteria, there is a clear correlation between age and thermostability for ancestral EF-Tu sequences: proteins from bacteria that lived during earlier periods are more heat stable than those from more recent times. This trend parallels the changes in temperature proposed for Precambrian oceans (which existed between 3,800 million and 542 million years ago) on the basis of analyses of the isotopic composition of Precambrian cherts. These cherts are some of the best-preserved sedimentary rocks from the period. It has been shown that the $^{18}O$ and $^{30}Si$ isotopic contents of Precambrian cherts are best explained by a progressive cooling of the oceans — from temperatures of about 70 °C some 3,500 million years ago to 20 °C just 800 million years ago[2,3].

But ancestral protein-sequence estimation is dogged by several uncertainties. The first problem is that such estimates are based on a phylogenetic tree that models evolutionary relationships between organisms, but in which the relationships between large bacterial groups are unconfirmed. The second issue is that statistical models of protein-sequence evolution depend on a simplifying hypothesis, which states that the probability that each amino acid will be replaced by another never changes with time. This may not be true. Finally, for any given tree and evolutionary model, billions of ancestral sequences are possible. But Gaucher and colleagues' results[1] are robust. They performed a series of control experiments that convincingly show their results to be qualitatively insensitive to the intrinsic uncertainties of their technique.

Other procedures for estimating ancient environmental temperatures from extant

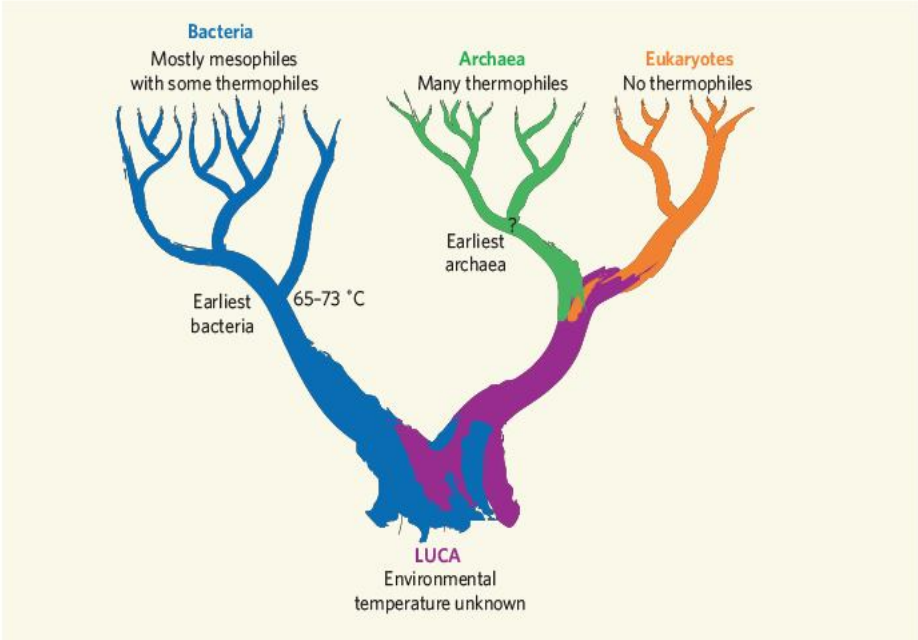


**Figure 1 | Environmental temperatures at pivotal points in early evolutionary history.** The tree of life stems from the last universal common ancestor (LUCA), which evolved into bacteria, archaea (microorganisms similar to bacteria) and eukaryotes (organisms with nucleus-containing cells). Extant bacteria are mostly mesophilic (they thrive at temperatures of about 25–40 °C), but some are thermophilic (preferring temperatures greater than about 60 °C). Many extant archaea are thermophilic, but no such eukaryotes exist. Gaucher *et al.*[1] modelled and reconstructed proteins from ancestral bacteria, and from their heat stability conclude that the earliest bacteria lived in oceans at temperatures of 65–73 °C. Previous studies[4,5] based on models of ancient RNA suggest that LUCA was either mesophilic or thermophilic. The temperature at which the earliest archaea flourished has not been estimated.

molecular-sequence data are known. For example, the amounts of the nucleic-acid bases guanine and cytosine found in certain RNA sequences of modern-day bacteria and archaea (a group of microorganisms similar to bacteria, but which evolved separately) strongly correlate with environmental temperatures. So by determining these RNA sequences for ancestral microorganisms, the temperatures of ancient habitats can be estimated (Fig. 1). But this method has yielded conflicting results, suggesting that very ancient life-forms were hyperthermophilic[4] but also that the last universal common ancestor (LUCA) of all modern life was mesophilic[5]. Previous statistical estimations of protein sequences support the idea that LUCA was hyperthermophilic[6].

Some of these conflicts might result from the inadequacy of the hypotheses used in evolutionary models. Emerging statistical methods that account for variation in the probabilities of DNA- or protein-sequence mutations with time[7] should help in accurately estimating the features of ancestral protein sequences. The recently discovered correlation between environmental temperatures and the average content of seven specific amino acids in bacterial and archaeal proteins[8] also provides a promising opportunity for future research.

Gaucher *et al.*[1] propose two possible scenarios to explain the striking agreement between the thermostability of bacterial EF-Tus and ocean temperatures in the Precambrian. The first is that bacteria from the Archaean lived in hot oceans, and progressively adapted to cooler oceans. Alternatively, bacteria could have started out in hot springs or thermal vents before expanding their territory into the cooler, global environment.

Sadly, geological evidence that might discriminate between these possibilities is scarce — Archaean microorganisms are poorly preserved compared with those of the late Precambrian (2,500 million to 542 million years ago). Geologists have, however, found laminated, sedimentary structures that are 3,430 million years old in the Pilbara Craton of Western Australia. By comparison with modern analogues, these 'stromatolite' structures are interpreted as reefs constructed by cyanobacteria[9,10]. In addition, carbonaceous microstructures of a similar age (3,460 million years) have been found in bedded cherts from the Apex Formation of Western Australia[11]. These are most probably fossils of bacteria or archaea, although the evidence for this is still debated. Nevertheless, the existence of these stromatolites and of fossil-bearing cherts, and their moderate enrichment in $^{18}O$ and $^{30}Si$, favour the idea that ancient bacteria lived in hot oceans.

Matching the phylogenetic tree of life to the geological record remains a fundamentally difficult problem, because the tree is built from molecules whose early rates of evolution are unknown. The significance of Gaucher and colleagues' contribution[1] is that it might open up a new way to overcome this problem: using molecular data to provide information about ancient environmental features that could allow ancestral organisms to be dated through the geological record. ■

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DEVICE PHYSICS

# Update on 3D displays

Joseph W. Perry

**Static three-dimensional images are easy to make using holographic techniques. Moving pictures are more of a problem. A palm-sized, updatable display using a specially designed polymer could be a breakthrough.**

Moviegoers who crave that feeling of being 'inside' the action will welcome the news from Tay *et al.* on page 694 of this issue[1]. The authors report a development that brings this dream a step closer to reality — an updatable, three-dimensional display based on cheap, easy-to-process photorefractive polymer materials. The technology still has some way to go to maturity, but ultimately it's not just the cinéastes who could benefit: displays that can provide realistic three-dimensional images with a wide angular viewing range might also be used in military or medical contexts, such as the simulation of field situations or the guidance of keyhole surgery.

Three-dimensional (3D) visualization technologies have a long history[2]. Early 'stereoscopic' approaches — familiar from the 3D movie craze of the early 1950s — used 2D displays and special eye-wear with polarizing lenses or lenses of different colours, so that separate images were seen in each eye. Alternatively, two images were switched rapidly on and off, with viewing glasses shuttered in synchrony to make the display appear continuous.

That kind of eye-wear is cumbersome, and

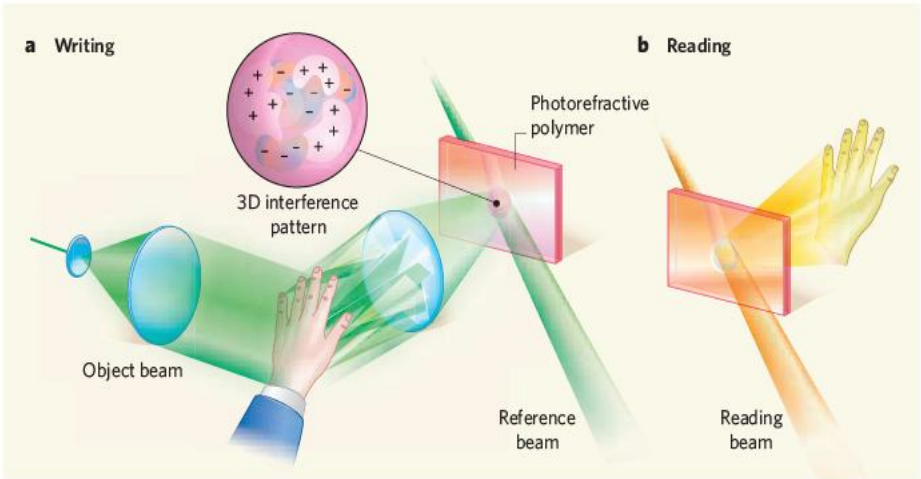


**Figure 1 | Handy display. a**, The base medium of Tay and colleagues' updatable, three-dimensional holographic display[1] is a photorefractive polymer onto which the 3D interference pattern of light scattered by an object and a reference laser beam can be etched volumetrically. In brighter regions of the interference pattern, mobile charge carriers — electrons (–) and 'holes' (+) left by departed electrons — are generated and the more mobile holes drift into the darker regions. The electric-field distribution, and so the local refractive index, of the medium are thus altered in a way that maps the amplitude and phase information of the light from the imaged object. **b**, When light from a second, reading laser beam illuminates the polymer, it is scattered in the same way as light on the original object, and a 3D image results. As the writing mechanism depends solely on the dynamics of charge carriers in the polymer, which in turn (for a given polymer and applied voltage) depends only on the incident interference pattern, the display is in principle fully updatable.

colour filters in particular can cause headaches if used over a long period. As a result, polarizing stereoscopic displays have not spread much beyond scientific applications. The development in the past decade of improved 'autostereoscopic' displays that are viewable with the naked eye, in which a 2D display equipped with a lens array brings different images to each of the viewer's eyes, creating an illusion of depth, has renewed interest in 3D imaging[3]. But these displays require the viewer to be situated at the correct distance from the screen to obtain the stereo effect. Multi-view displays, or head-tracking displays that correct the image for the viewer's location, are some help, but increase the cost of making, processing and projecting the images.

Holography is another well-known 3D display technology, and provides high-resolution views over wide angles[4]. Holographic displays are autostereoscopic, in that the 3D image is 'stored' in a material and can be viewed when illuminated with no need for glasses, lens arrays or other devices. They work by recording both laser light scattered from the object to be imaged and a plane-wave laser reference beam to form an interference pattern that is stored in 3D in, for example, a photopolymer. In such polymers, light in the interference pattern activates a chemical reaction that locally modifies the material's refractive index. This process stores all the optical amplitude and phase information needed for 3D image projection, but cannot be repeated: the image on the display is static.

Tay and colleagues' breakthrough[1] is to demonstrate a reasonably sized display, measuring 10 cm × 10 cm, whose base medium is what is known as a photorefractive material. Photorefractive materials also capture image (amplitude and phase) information in 3D. But unlike photopolymers, they are dynamic storage media: information can be stored, erased and rewritten (Fig. 1).

This kind of photorefractivity has been studied extensively in crystals of inorganic oxides such as lithium niobium oxide ($LiNbO_3$; ref. 5). In these materials, the absorption of a holographic interference pattern leads to the generation of mobile charge carriers in bright regions of the interference pattern. Under an applied field (or, in a polar crystal, the internal field), the mobile charges drift and accumulate in the dark regions. The result is a change in the electric-field distribution, and resultant, proportional changes in the crystal's local refractive index through a phenomenon known as the electro-optic effect. Inorganic crystals are known to be extremely photosensitive, and images of high resolution can be produced in this way. Unfortunately, growing crystals of a large enough area for practical displays is both difficult and costly.

This is where photorefractive polymers[6] become interesting: they possess all the capabilities of the inorganic crystals, but can be processed to produce large-area displays using low-cost, solution- or melt-based methods. The photorefractive polymer developed by Tay *et al.*[1] contains a copolymer combining a molecular group that transports positive charge with a dye whose polarization changes nonlinearly in response to light. The dye rotates and aligns under the influence of an applied electric field, modifying the material's refractive index. The copolymer also acts as a photosensitizer, absorbing light and generating mobile charges at the 'writing' wavelength of 532 nanometres.

The material thus developed possesses a remarkable combination of the properties crucial to a photorefractive display. Its optical quality and homogeneity are improved by use of the copolymer to minimize phase separation (the separation of dissimilar components of the material, forming regions that scatter light, degrading the image quality). High photosensitivity, allowing the use of only moderate laser powers for writing and erasing, is assured by adding small amounts of both a fluorinated molecular group with a nonlinear optical response and a plasticizer to promote molecular motion. A diffraction efficiency of close to 90% leads to good display brightness and low read-out power.

Operating their display under an applied voltage of 9 kV during writing, to speed up charge transport, but 4 kV for read-out, the authors could write across the full area of the display in about 3 minutes and hold an image on it for up to 3 hours. A fully automated optical system processed the object beam into strips that were recorded sequentially in adjacent parts of the display, using a laser intensity of around 100 milliwatts per square centimetre and a good writing speed. The principle of operation is entirely scalable: with higher-power lasers or more sensitive photorefractive polymers, larger areas could be written at the same time, or a small area faster.

Future advances in the size and speed of updatable holographic 3D displays would create a powerful and high-resolution visualization technology. But there is fierce competition, driven by the size of the potential military, medical and entertainment markets, with large-area, flat-panel 3D displays and alternative real-time 3D displays that are coming on in leaps and bounds. For film fans and gamers itching to be in the midst of the action, the wait might not be too long. ■

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## 50 YEARS AGO

During the past few months, three great achievements in science and technology have justifiably received world acclaim: (1) the launching of artificial Earth satellites by scientists in the U.S.S.R.; (2) the development and control of thermonuclear fusion by scientists in Great Britain and the United States; (3) the launching of an artificial Earth satellite by scientists in the United States. We have used the expression 'by scientists in' deliberately... Scientific discoveries are not suddenly made by men who, by birth or consent, are entitled to claim a certain nationality; they are the results of good training, patient and sometimes long-continued work often fraught with disappointment, team-work sometimes spiced with real personal genius, and all these are based on the work of previous generations.

From *Nature* 8 February 1958.

## 100 YEARS AGO

Mr Mallock (January 30, p. 293) seems to presume, as a great many others do, that an apparatus on the aëroplane principle "demands constant attention on the part of the aëronaut" to maintain its stability in the air. We are apt to get ideas from watching the behaviour of little bits of paper floating in the gusts of wind, and to forget that the flying machine of the future may run into tons of weight. Though a frail canoe may easily capsize, the big ship seldom turns over even in the roughest of seas. Even so primitive a contrivance as we may presume that of Mr. Farman to be is some 33 feet across and weighs, complete, half a ton. Such a structure is not easily upset by mere puffs of wind. But it is also evident that a machine can be designed possessing nearly perfect automatic stability... A well-designed and well-balanced machine is automatically stable without any pendulums or other appliances; in fact, it forms a pendulum of itself.

**B. Baden-Powell**

From *Nature* 6 February 1908.

50 & 100 YEARS AGO

NEUROPATHOLOGY

# Alzheimer's in real time

Eliezer Masliah

**A hallmark of Alzheimer's disease is the presence in the brain of protein deposits, or plaques, which are thought to form over a long period. But studies in mice suggest that the plaques can grow overnight.**

Alzheimer's disease is the commonest neurodegenerative disorder in ageing human populations. It is characterized by memory loss and behavioural alterations in its early stages, and by severe cognitive impairment and dementia later on. Alzheimer's is diagnosed definitively only after death, through the identification in the brain of extracellular protein deposits known as amyloid plaques and intracellular protein aggregates called neurofibrillary tangles. It is unclear exactly when and how the plaques form, but classical neuropathological studies have indicated that they develop over extended periods of time and long before the dementia becomes apparent. Reporting in this issue, however, Meyer-Luehmann *et al.* (page 720)[1] provide surprising evidence that, in a mouse model of Alzheimer's disease studied in real time, amyloid plaques form extremely rapidly — within 24 hours.

Amyloid plaques form through the extracellular accumulation of polymers of the amyloid-β protein, which is a breakdown product of the amyloid precursor protein. Abnormal, tortuous neuronal processes surround the plaques, and an inflammatory reaction involving the brain's support cells, astroglia and microglia, further dramatizes the scene[2].

To study the kinetics of plaque formation, Meyer-Luehmann *et al.* used state-of-the-art, multiphoton laser confocal microscopy, which is an innovative way to visualize tissues *in vivo*[3]. Compared with conventional microscopy tools, which have been used extensively to study amyloid plaques, the main advantage of the multiphoton technology is that it allows both optical sectioning of the tissue and deep visual penetration into it. Thus, without physically sectioning and chemically fixing a tissue, the investigator obtains three-dimensional, real-time information from an intact brain and, more importantly, a live animal.

Using this technique, the authors[1] observed remarkably different kinetics of plaque formation from that expected: new plaques formed in only 24 hours, and their size and final characteristics stabilized within a week (Fig. 1). Stable plaques were also reported in an earlier study[4] showing that, in another mouse model of Alzheimer's disease, reducing amyloid-β production might halt disease progression, although existing plaques persist.

A question that has long puzzled neuropathologists is how the initial lesion forms from which the plaques develop. Earlier studies[5] involving conventional imaging of fixed tissues suggested that larger, diffuse or amorphous amyloid deposits might be the nidus for the mature plaques. But Meyer-Luehmann *et al.* describe a very different picture in which the mature plaques originate from smaller amyloid deposits (microplaques) that support a fast but eventually stable growth of the plaques. The origin of the microplaques is unclear, but it is possible that submicroscopic aggregates are present in the brain, perhaps related to the presence of small assemblies (oligomers) of amyloid-β molecules that may act as precursors to this rapid and sudden growth.

Among the features of the neurodegenerative process in Alzheimer's disease are damage to the synaptic connections between neurons, neuritic dystrophy — the formation of tortuous neuronal processes — and, eventually, loss of selective groups of neurons. Such damage to synapses and axons in specific neuronal populations has been strongly linked[6,7] to the cognitive impairment seen in Alzheimer's disease. But the mechanism that causes the damage is a matter of great debate. One contentious suggestion[8] is that synaptic damage is linked to the accumulation of neurotoxic amyloid-β oligomers rather than to that of the longer fibrils.

As clinical studies[6,7] show that plaque abundance is a poor indicator of the severity of dementia, some investigators have questioned the pathogenicity of these lesions. But Meyer-Luehmann *et al.*[1] show in mice that, in the early stages of the disease, microplaques can damage neighbouring axons and dendrites within

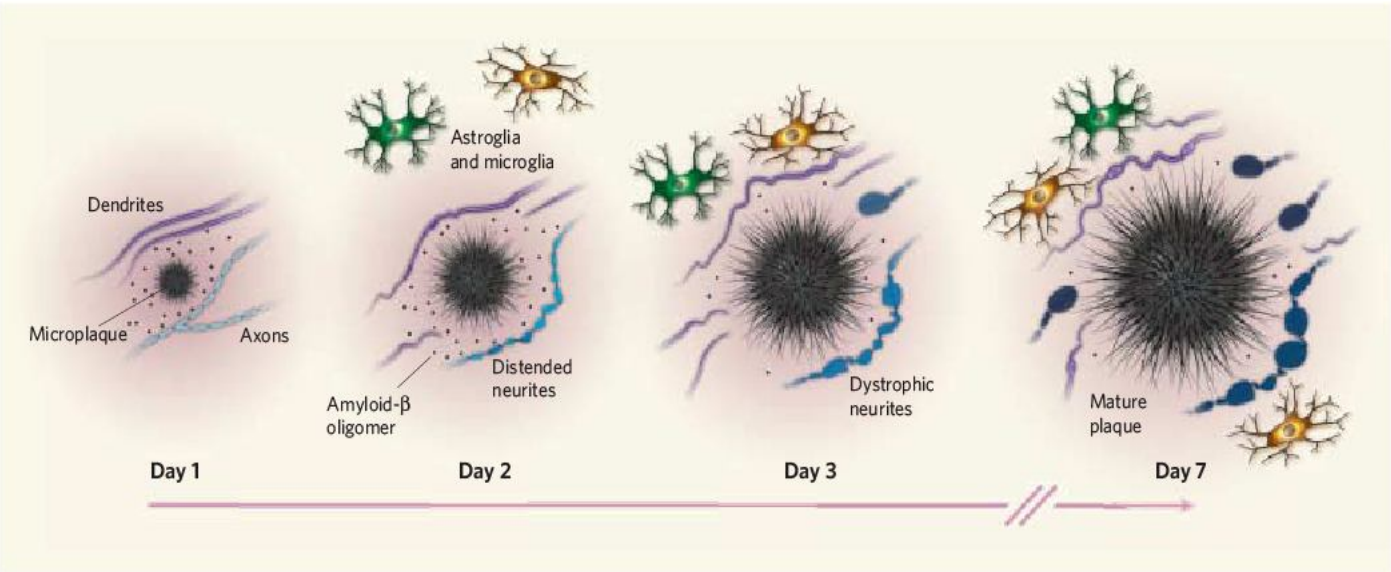


**Figure 1 | From plaque inception to maturity.** Meyer-Luehmann *et al.*[1] studied a mouse model of Alzheimer's disease, monitoring 5–6-month-old animals — the age at which they begin to form the plaques characteristic of the disorder. The day on which the authors first detected a small extracellular amyloid deposit, or microplaque, was designated day 1. At that time, there was minimal alteration in the neurites surrounding the microplaque. But by day 2, the amyloid deposit had grown rapidly, and alterations in neighbouring axons and dendrites had become apparent. Migration of support cells, such as astroglia and microglia, to the vicinity of the growing plaque had also begun. By day 3, frank damage to the neighbouring axons was apparent. By day 7, the plaque had reached maturity, and its structure had stabilized.

days. This observation hints that the microplaques might interfere with the transport of organelles and molecules along the axon, perhaps resulting in neuritic dystrophy. Moreover, in the vicinity of the microplaques, amyloid-β oligomers might also contribute to neuritic dystrophy. Further studies are necessary to clarify the exact mechanisms behind the pathogenicity of the plaques, and the extent to which the associated neuritic dystrophy is linked to the dementia seen in Alzheimer's.

Meyer-Luehmann and colleagues' findings are limited to two animal models of the disease, and their validity should be verified in other models. Nonetheless, in their use of an innovative technique for studying lesions, the authors have opened a new avenue in neuropathology that can be extended to investigate the kinetics of other lesions (such as neurofibrillary tangles, the Lewy bodies in Parkinson's disease and the intranuclear inclusions in Huntington's disease), and to evaluate synaptic activity and response to treatment in living tissues. ■

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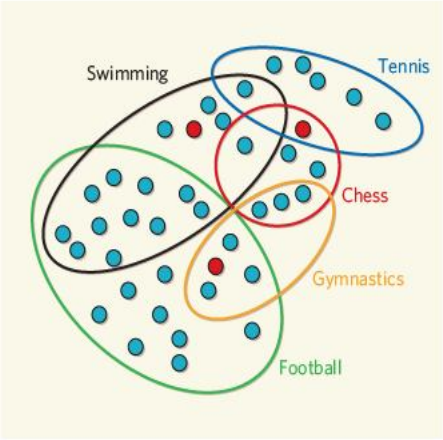


**Figure 1 | Hitting sports.** In this representation of a simple hitting-set problem, students (dots) play one or more sports (encircling sets). The hitting-set problem asks what the smallest set of students is that encompasses all sports — in this case, the size of this set is three (red dots). The hitting-set problem is a classic example of an NP-complete problem, in which the complexity (and thus computing time and power needed to find a solution) probably grows exponentially as the numbers of variables (students) and constraints (sports) increase. Mézard and Tarzia[3] show how the statistical methods used to characterize the interactions of ensembles of many particles in fundamental physics can be applied to solving hard, random instances of the hitting-set problem with thousands of variables and constraints.

COMPUTATIONAL SCIENCE

# A hard statistical view

Bart Selman

**The sheer number of variables and logical conditions makes some computing problems seem intractable. Statistical physics, normally used to study huge groups of interacting particles, can supply powerful tools to crack them.**

As computer hardware and software become ever more sophisticated, we are shifting from a setting in which computers merely assist us in processing information with the aid of well-understood algorithms, to a landscape in which computers themselves make decisions and are in full control of a given situation. An example is the Pentagon-sponsored 'Darpa Urban Challenge'[1], in which standard consumer vehicles are equipped with sensors such as laser range-finders, cameras and global positioning systems. Taking millions of measurements per second, and using up to a dozen PCs to process the data, these vehicles can make driving decisions on their own in real time.

Such computational tasks are fiendishly complex. Many of them in fact fall into a general class of notoriously hard computational problems known as NP-complete problems. (The NP stands for 'non-deterministic polynomial time; the general assumption is that, in the worst case, the time needed to solve NP-complete problems explodes exponentially with the number of variables.) First characterized[2] in the early 1970s, thousands of these problems have since been identified, in areas as diverse as hardware and software verification, planning and scheduling, automated reasoning, and computational biology. Writing in *Physical Review E*, Mézard and Tarzia[3] demonstrate an innovative approach to solving one well-known NP-complete problem, known as the hitting-set problem. Their approach borrows techniques from the statistical physics generally used to characterize the interaction of atomic ensembles, and highlights a trend of recent years: the application of fundamental physics to bring new perspectives to the study of hard computational problems.

The hitting-set problem starts with a number of sets, each containing a certain number of items. Each individual item can occur in more than one set. A hitting set 'hits' the original sets in that it contains at least one item from each; the challenge is to find the smallest such set. Take a class of students, for example, each of whom plays one or more sports (Fig. 1). For each sport, there is a group (set) of students playing the sport. Here, the question would be: what is the smallest group of students I can choose so that all sports are represented in my sample?

Hitting-set problems arise in many contexts, including fault diagnosis, group testing (for example, analysing many blood samples by analysing a few aggregates of individual samples), database searches, experiment design and DNA screening. The difficulty, in computational terms, is that the number of combinations of items that needs to be considered to find the smallest possible hitting set, or a hitting set smaller than a certain size, grows exponentially with the number of items. Such exponential or combinatorial search spaces are characteristic of NP-complete problems.

In a broad sense, such problems involve a set of discrete variables and a set of constraints between the variables that model their interactions[4]. In terms of our earlier hitting-set example, each student is represented by a binary variable, which is set to 'one' if the student is part of the hitting set, and otherwise set to 'zero'. Each sport introduces a new constraint on the variables: at least one of the students playing a particular sport should have a variable set to one. A further constraint places an upper bound on the number of variables set to one, and therefore on the size of the hitting set. Finding variable assignments that satisfy individual constraints is generally quite easy. The challenge is to find an assignment to the variables that satisfies all constraints simultaneously.

Algorithms for doing this fall into two broad classes: backtrack search, introduced in the early 1960s, and local search, which popped up a decade later. Backtrack search methods proceed in a centralized way, by assigning values to variables one by one. Whenever a local constraint is violated — when none of the students playing a certain sport is flagged as belonging to a hitting set — one or more variable settings is revisited and changed. Simple book-keeping techniques ensure that such a search explores the entire combinatorial search space.

In local search methods, by contrast, the exploration is less systematic. First, with a random guess, one assigns values to all variables. Such an assignment will generally violate

many constraints, and a local search algorithm proceeds by trying to 'fix' variable settings to reduce the number of violations in the search for a variable setting that satisfies all constraints.

In tackling the hitting-set problem, Mézard and Tarzia[3] follow a fundamentally different route. They take advantage of a significant advance that occurred in the early 1990s, when computer scientists banded together with physicists to study ensembles of randomly generated instances of various NP-complete problems[5–8]. An 'instance' here is simply a particular example of the generic problem, defined by a set of variables, and specific governing constraints; in our previous example, one specifies a set of students and the sports they play.

This work revealed that, at certain values of the ratio of constraints to variables, ensembles of random instances of the same generic problem underwent a sudden change, dubbed a phase transition. Below the phase-transition point, most instances have one or more solutions that satisfy all constraints; above the phase transition, most instances do not have any solution, because there are too many constraints to satisfy. The instances that were hardest to solve occurred with numbers of variables and constraints that lay exactly at these phase-transition boundaries. A natural conclusion was that tools from statistical physics developed to study physical phase transitions might help in developing more efficient algorithms for solving combinatorial problems.

An example is the 'survey-propagation method'[9] used by Mézard and Tarzia[3], which developed from the cavity method used in statistical physics to calculate ground-state properties of certain condensed-matter systems. Survey propagation solves random instances of the boolean satisfiability problem near phase transitions with large numbers of variables (more than $10^7$), which are beyond the reach of backtrack and local-search methods. This archetypal NP-complete problem asks the question of whether, given a set of logical statements using boolean variables (variables that can be either 'true' or 'false'), there is any assignment of values to those variables that can satisfy all the statements.

Mézard and Tarzia use the survey-propagation method to compute statistical properties of the solutions of instances of the hitting-set problem. Such a strategy might seem doomed to fail because it is generally significantly harder to determine the properties of the set of solutions of a hard computational problem than it is to find a single solution. But survey propagation can efficiently approximate the requisite statistical information for instances of various combinatorial problems near phase boundaries. It does this by iteratively solving a large set of coupled equations, modelling the local interactions between variables probabilistically. This solution process can be performed in a parallel, distributed fashion using many different processors, and generally converges to an answer extremely quickly — in seconds for equations with thousands of variables.

Survey propagation can be viewed as a generalization of the 'belief-propagation method'[10], which was discovered independently in several fields, including information theory and artificial intelligence. Belief propagation is a way of approximating the probability (the 'belief') that a variable takes on a particular value in a randomly sampled solution. This information can be used to set variables incrementally, thus simplifying a problem.

The method works well when solutions are nicely clustered together in the combinatorial space, which is the case reasonably far from a phase transition. Near phase boundaries, however, solutions break up into many smaller, unconnected clusters in the combinatorial space[11]. Conventional combinatorial search algorithms and standard belief-propagation techniques become trapped between these clusters, and cannot effectively search the solution space. The survey-propagation method, on the other hand, continues to provide reliable statistical information about the solution space[12–14].

It is this property that allows Mézard and Tarzia[3] to map out for the first time the space of hitting-set problems, identifying under what conditions belief- and survey-propagation methods can solve hard, random instances of the problem. They also identify regions where still more complex survey-propagation-style equations would be required.

Given the ever increasing role of computational methods in other disciplines, the fact that those other disciplines are, in turn, starting to contribute new concepts and ideas to the science of computation is an exciting development — one that, as the demands we make on computational methods continue to grow, we are sure to hear more of. ■

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EVOLUTIONARY GENETICS

# Who shouldn't be your daddy

Patrick C. Phillips

**Unusual reproductive incompatibility has been discovered between two strains of a nematode worm. This finding indicates that natural selection can generate long-term divergence within self-fertilizing populations.**

Reproductive incompatibility is the stuff of speciation and lies at the heart of the world's tremendous biodiversity. It makes little sense for such incompatibility to be maintained within a species, however. After all, what is the advantage of having genes that kill your offspring? As they report in *Science*, Seidel and colleagues[1] attempt to answer this question by identifying two genes that mediate reproductive incompatibility between different populations of the nematode worm *Caenorhabditis elegans*.

The authors crossed two evolutionarily divergent worm lines, one from Bristol, UK, and the other from Hawaii, and analysed the genome of the resulting offspring for genetic markers — known DNA sequences that differed between the two strains. They noticed that, rather than the expected one-to-one ratio of Bristol and Hawaii markers, nearly all of the markers on one specific region of chromosome I were of the Bristol type.

To obtain second-generation offspring from the Bristol–Hawaii hybrid, Seidel *et al.* crossed the hybrids with one another and found that a quarter of the resulting embryos died. Remarkably, nearly all of the dead embryos carried Hawaiian genetic markers in the same region of chromosome I.

To narrow this effect down, the authors again constructed hybrids between the two strains and then crossed them back to the Hawaiian strain (Fig. 1). The mating system of *C. elegans* is unusual in that this worm can occur as a male or as a self-fertilizing hermaphrodite. The authors found that when eggs of Hawaiian worms were fertilized by sperm from a Hawaii–Bristol hybrid (either a male

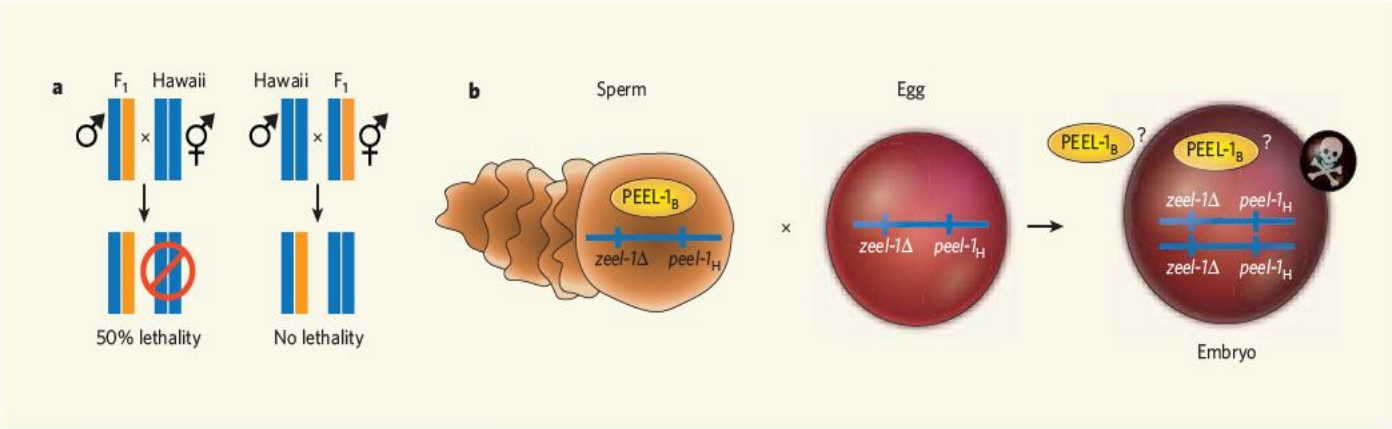


**Figure 1 | Worms like to keep it in the family.** **a**, Seidel *et al.*[1] crossed strains of *Caenorhabditis elegans* from Bristol and Hawaii. They then mated the hybrid male offspring of this cross with the Hawaiian strain. This led to a high proportion of embryonic lethality (50%) in the second-generation offspring. If, however, the sperm donor was a worm of the Hawaiian strain crossed with a Bristol–Hawaii hermaphrodite, no lethality was observed. **b**, Two genes are responsible for reproductive incompatibility between these divergent strains. The first, *peel-1*, is a paternally acting factor and the second is *zeel-1*, the expression of which is necessary in the embryo to counteract the lethal effects of Bristol *peel-1* product (PEEL-$1_B$). In the Hawaiian strain, *zeel-1* is missing entirely (*zeel-1*Δ). Here PEEL-$1_B$ expressed by sperm precursor cells is transferred to the sperm containing the Hawaii copy of the incompatibility region (*zeel-1*Δ, *peel-*$1_H$). The lethal outcome is observed when an embryo with the *zeel-1*Δ locus is generated from a sperm containing PEEL-$1_B$. It is not known whether PEEL-$1_B$ acts within or outside the embryo.

or a hermaphrodite), the embryos died. But if the Hawaii–Bristol offspring was the 'mother' (or egg donor) and a Hawaiian worm donated the sperm, the embryos survived (Fig. 1a). The authors therefore speculated that the first-generation Hawaii–Bristol offspring must carry a paternally acting gene in the Bristol-type genomic region of chromosome I — what they call the incompatibility region — that is responsible for embryonic lethality.

Post-fertilization effects of paternally expressed genes are quite rare because the sperm is thought to primarily transfer only its DNA to the developing embryo and few products originating from gene expression within the father (such as proteins or messenger RNA). Therefore, to test their hypothesis, Seidel *et al.* carried out further crossing of the inbred worm lines and narrowed down the incompatibility region to a 62-kilobase segment of nucleotides. Here they identified two new genes — *zeel-1*, which acts in the one-cell embryo, and *peel-1*, which is the paternal-effect factor from the sperm.

The locus (position) in which *zeel-1* occurs is missing in the Hawaiian strain. So the authors suggest that the incompatibility between Hawaii–Bristol hybrids and Hawaiian worms must be due to the action of the product of the paternal, Bristol *peel-1* in the absence of 'maternal' *zeel-1* (which would otherwise counteract the lethal effects of *peel-1*)(Fig. 1b).

Next, to gain insight into possible speciation in *C. elegans*, Seidel *et al.*[1] investigated the worldwide distribution of *zeel-1* and *peel-1* in natural isolates of this nematode. They found that both of these genes seem to be widely distributed, and can even occur in the same population. This is no way for an incompatibility system to behave! A possible explanation for this pattern could be recent, possibly human-assisted, broad-scale migration[2] of *C. elegans* populations. Consequently, variation in the geographical distribution of *zeel-1* alleles (copies) could be due to mixing between previously isolated strains. Seidel and colleagues, however, provide strong evidence against this view.

If this sort of mixing were to have occurred recently, we should see co-inheritance of large blocks of the genome in different strains. Instead, the authors find that, outside the incompatibility region, the Hawaiian and Bristol strains are genetically almost identical, and only within this region are there large differences in nucleotide sequence — differences that are more than 50-fold greater than usually found among *C. elegans* isolates. Seidel *et al.* take this high degree of polymorphism to mean that the *zeel-1* and *peel-1* loci are quite ancient and predate the divergence times of the surrounding genomic regions. This indicates that the incompatibility region has introgressed — infiltrated through repeated mating between different strains — into the genomes of many strains, and that this introgression has presumably been occurring on a worldwide scale for a long time.

What evolutionary forces might generate such a pattern? The authors offer two possibilities. First, because of the lethality induced by *peel-1* in worms not also carrying *zeel-1*, the Bristol *zeel-1* allele has a strong transmission advantage from the death of embryos with a Hawaiian background. The close proximity of *zeel-1* and *peel-1* loci is reminiscent of similar systems seen in the fruitfly *Drosophila* and other organisms[3] that allow, for instance, sperm of one type to prevent fertilization by sperm of another type. But alleles with this kind of advantage are thought to become quickly fixed within populations and not to result in the form of ancient polymorphism seen here.

Although it is possible that selection for the Bristol *zeel-1* allele is precisely counterbalanced by selection operating in the opposite direction on the Hawaiian allele, Seidel *et al.* dismiss this as highly unlikely. Instead, they favour the interpretation that polymorphism in these two genomic regions is maintained by balancing selection, which favours allele diversity, with the incompatibility that arises as a by-product of the long-term divergence generated by this selection. The most likely form of balancing selection in this case would be some kind of frequency-dependent selection in which rare variants are favoured, as is commonly observed in pathogen-resistance systems.

Why the incompatibility, then? This is where the unusual mating system of *C. elegans* (males and hermaphrodites) comes into play. Hermaphrodites can only self-fertilize, and so inter-crossing of animals of different strains occurs only through males, which are thought to be quite rare in many natural populations[4,5]. If most individuals are self-fertilizing, these divergent alleles can be maintained for a long time with little threat from induced lethality because the alleles would come into contact only infrequently.

Seidel and colleagues' work[1] is important because it is one of the few examples in which a specific incompatibility system has been identified at the molecular level. But it is perplexing because what one might have expected to be an elegant case of incipient speciation is something quite different. At the very least, this work highlights the emerging picture that genomic evolution in *C. elegans* is strongly dominated by its self-fertilizing mode of reproduction. ■

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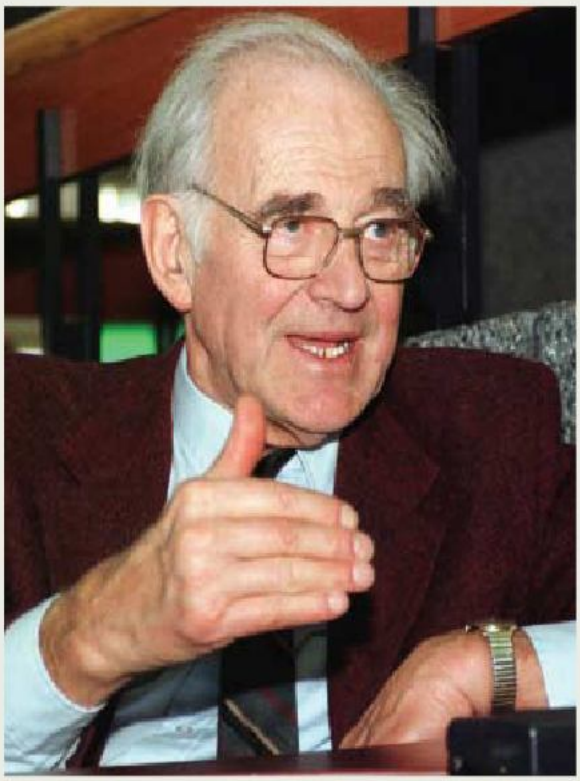
OBITUARY

# Bert Bolin (1925–2008)

Pioneering climate scientist and communicator.



K. KASAHARA/AP

As the first chair of the Intergovernmental Panel on Climate Change (IPCC), and one of the first scientists to understand the environmental impact of carbon dioxide produced by human activities, Bert Bolin left an indelible mark. A pioneer of climate science, he died in Stockholm on 30 December 2007, aged 82.

Bolin was born in Nyköping, Sweden, on 15 May 1925. He completed his PhD at the University of Stockholm in 1956, and was within five years professor of meteorology there — a post he held until his retirement in 1990. During that time, he published more than 160 papers related to the meteorology and chemistry of the atmosphere, contributing to an improved understanding of numerical weather models and acid deposition.

As early as the 1950s, Bolin started to study the natural carbon cycle. His fundamental research advanced our understanding of the fate and transformations of carbon dioxide, not only in the atmosphere, but also in the oceans and in the terrestrial biosphere. He was among the first scientists to recognize the significance of changes in ecosystems for atmospheric carbon dioxide, and that deforestation in particular was contributing to the observed increase. He was also one of the first to go public with his concerns: in May 1959, he travelled to Washington DC to warn the National Academy of Sciences that a 25% increase in carbon dioxide in Earth's atmosphere by the end of the century could have serious consequences for the temperature of the planet.

Bolin rapidly acquired a reputation as an eminent organizer and leader of cross-border scientific collaborations. In 1963, he became involved in setting up an international effort to study the general circulation of the atmosphere. This work led to the formation of the International Council for Science's (ICSU's) committee on atmospheric sciences in 1964, of which Bolin became the first chair. That committee's work resulted in the establishment three years later, by ICSU and the World Meteorological Organization, of the Global Atmospheric Research Program — GARP.

The timing of this move was especially significant: the availability of the first information on Earth from space was exciting meteorologists across the world, owing to the unprecedented opportunity it offered to study the atmosphere as a whole. This ambitious goal was supported by the rapidly growing potential of computers to perform large-scale modelling. Bolin chaired GARP from 1968 to 1971, bringing together scientists from around the world at the height of the cold war. Under his aegis, GARP became an acclaimed international research programme that contributed much to our understanding of weather and climate.

In 1983, Bolin began a project supported by the United Nations Environment Programme to explore the links between the physical climate system and global ecosystems. The result was the foundation, under the auspices of ICSU, of the International Geosphere–Biosphere Programme, which brought about a new level of integration between physical, chemical and biological perspectives of the global ecosystem. Bolin's particular insight was to comprehend the magnitude of the problems faced by the scientific community in working across disciplinary boundaries, as well as to envisage how these problems might be solved.

These were qualities that served him well in the role for which he will undoubtedly be best remembered — as the first chair of the IPCC, from 1988 to 1998. His reputation as a brilliant and honest scientist, who listened to and respected diverse views, attracted the best and the brightest of the scientific community to the IPCC, and the fledgling panel rapidly gained the attention of the politicians to whom its reports were addressed. Bolin's quiet, soft-spoken style earned him the trust and respect not just of government officials who already recognized the threat of human-induced climate change, but also of those who vehemently challenged the idea that Earth's climate was even changing, let alone whether humans were involved. These same 'soft' skills helped him nurture talent in his own research team in Stockholm, where he was a mentor to many young researchers who have since become leading climate scientists.

Rarely does a single individual change the world, but Bolin's work as a scientist, as an organizer of major international research programmes and as leader of the IPCC has certainly changed the way we think about the world. That we are now aware of the potentially catastrophic impact of human activities on Earth's climate, and of the need to make the transition to a low-carbon economy and to protect our natural forests, is in no small part down to him.

Without his leadership of the IPCC, the 1992 Rio de Janeiro Framework Convention on Climate Change and the 1997 Kyoto Protocol would have taken longer to negotiate. His vision was central to all of the achievements for which the IPCC was jointly awarded the 2007 Nobel Peace Prize along with the former US vice-president Al Gore. Bolin was by then too ill to attend the ceremony but, as Gore wrote to him: "Bert, without you we would not have come to where we are today."

Brilliant yet humble, Bert Bolin was an excellent communicator, a leader despite his natural shyness, and one who always gave credit to others rather than to himself. A world-class scientist, a man of great integrity, a great organizer and an inveterate optimist, he was above all a nice guy who just enjoyed singing in his choir at home in Sweden. Isaac Newton famously said that we in science all stand on the shoulders of giants. For those of us who knew and worked with Bert at the IPCC, he above all was the giant upon whose shoulders we stood.

**Bob Watson**

Bob Watson was Bert Bolin's successor as chair of the IPCC. He is currently at the University of East Anglia, Norwich, UK, and is chief scientific adviser to the Department for Environment, Food and Rural Affairs, Nobel House, 17 Smith Square, London SW1P 3JR, UK.
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# HORIZONS

A. BARKER/ALAMY

When *Nature* asked a group of experts to offer their visions of the future, we were aware that such a project can have its pitfalls. Experts can get things drastically wrong — although, as Arthur C. Clarke noted, this usually occurs when they assert what is *not* possible. When they say what is possible, they can be inspiringly right.

With such inspiration in mind, these five Horizons articles offer a sense of what our authors believe should happen over the next few years. The collection is in no way comprehensive — we simply wanted to deliver a mix of fundamental and applied science, with the writers articulating their unrefereed agendas for their disciplines.

So it is that one article examines the advantages of a systems approach to researching human ageing. In a similar spirit, other authors show how we might develop the batteries to power the computers and transport of the future. The complex interactions of light and of matter in electronic structures and their potential to revolutionize future computation and communication are also explained. Another article shows how fossil evidence, genomic sequencing and molecular developmental biology should reveal more about how we humans came to evolve into what we are. And the description of the next generation of the web, in which computers can make as much use of information as humans can, offers a truly collaborative vision for research empowerment.

I hope that these visions will inspire and maybe even encourage some to adjust their research ambitions as a result. Inspired by them ourselves, we'll be publishing more Horizons in the future.

**Philip Campbell, Editor-in-Chief, *Nature***

R. RUNNING/SOLUS-VEER/CORBIS

# A systematic look at an old problem

Thomas B. L. Kirkwood

**As life expectancy increases, a systems-biology approach is needed to ensure that we have a healthy old age.**


The continuing increase in life expectancy, which in many countries advances by several hours per day, is one of humanity's most astonishing successes. But as the population ages, new approaches are required to unravel the complex biology of ageing and understand its links with frailty and disease.

The increase in human life expectancy over the past ten years has taken both scientists and the population generally by surprise. Until recently, demographers were confidently predicting that once the gains made by reducing mortality in early and middle life had reached completion, growth in longevity would stop and we would see the fixed reality of the ageing process. This has not happened[1]. In much of the developed world, life expectancy continues to increase at the rate of five or more hours per day; in some developing countries, which have some catching up to do, the rate is even faster.

Precisely why life expectancy is still rising (Fig. 1), and where this process might end, is some thing we need urgently to discover. Think of it this way. You woke up this morning to what is effectively a 29-hour day. Twenty-four of those hours, you will use now; the other five will be put by for later. The challenge posed by population ageing translates into ensuring that these extra hours will be as good as possible, free from high-cost dependency, when in time we come to use them.

Meeting this challenge requires research that is neither overly simplistic nor overwhelmed by the apparent complexities of the ageing process. Lord Rayleigh, the 1904 Nobel laureate in physics, asserted that one should "neither seek nor avoid complexity" in finding appropriate solutions to problems. This approach is the cornerstone of a long-term effort to tackle the challenge of an ageing population using an intensely multidisciplinary approach known as 'systems biology'[2] — essentially, the study of interactions between the components of a biological system (see Box 1). Ageing is a highly complex biological problem that benefits greatly from systems biology. Equally, ageing will surely demonstrate the worth of systems science.

Not all scientists have been ready to engage with the complexity of ageing, however. Some have suggested that ageing is too complicated for serious scientific study, or that it is like a slow-motion car crash — everything just gets wrecked. There are even those who declare, rather strangely, that "there is no such thing as ageing — old age is associated with disease, but does not cause it"[3].

At the opposite extreme are those who see ageing merely in terms of some favourite mechanism — a simple matter of the erosion of telomeres (the protective structures at the ends of chromosomes), oxidative damage by 'free radicals', or the dysfunction of mitochondria (the energy-generating organelles within the cell).

The discovery that single gene mutations can cause major increases in lifespan in animals such as the nematode *Caenorhabditis elegans* prompted many to think that the genetic control of ageing lay in a simple programme that evolved, perhaps, for the altruistic purpose of

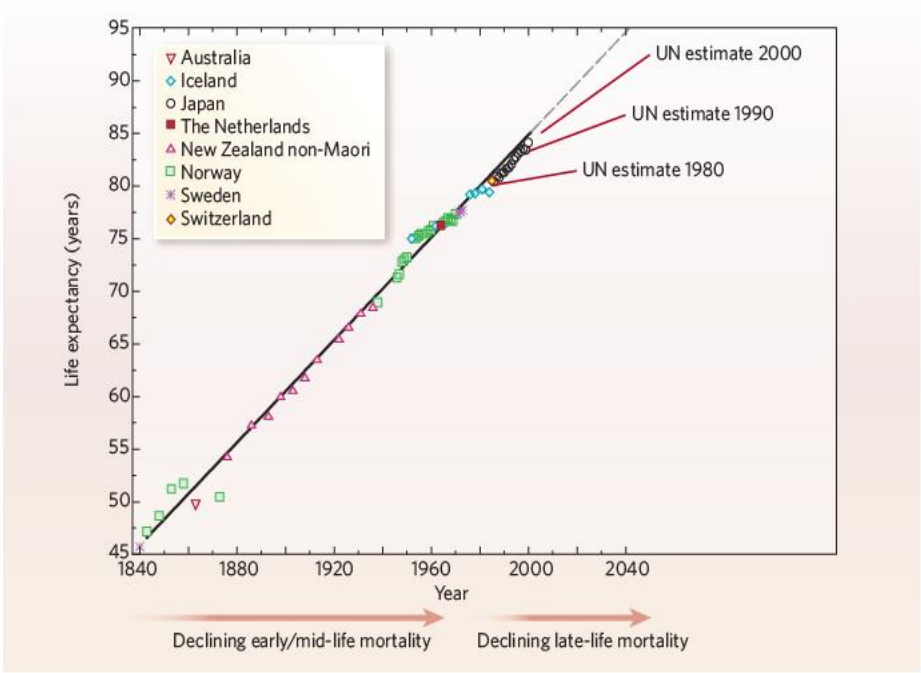


**Figure 1 | Life expectancy around the world has increased steadily for nearly 200 years.** The graph (adapted from ref. 1) shows the life expectancy in the then longest-living country. During the nineteenth and early twentieth centuries, the increase was driven mainly by improvements in sanitation, housing and education, causing a steady decline in early and mid-life mortality, which was chiefly due to infections. This trend continued with the development of vaccines and then antibiotics. By the latter half of the twentieth century, there was little room for further reduction in early and mid-life mortality. The continuing increase is due almost entirely to a new phenomenon: the decline in late-life mortality.

bringing life to a timely close, thereby creating necessary living space for progeny.

The idea that ageing is programmed was dismissed long ago by evolutionary gerontologists who recognized that natural selection could not, and would not, bring about such a fate (except in very special circumstances)[4,5]. Even for those who spurned such logic, the idea of programmed ageing began to seem rather odd when it was found that the genes that affect longevity do so not by changing the timing of a mechanism for self-destruction, but by adjusting hundreds of mechanisms for maintenance and repair[6,7]. If ageing were programmed, this would be a clumsy way to do it.

## Bridging the simple and the complex

Clear consensus now exists that ageing is caused by the gradual, lifelong accumulation of a wide variety of molecular and cellular damage[8]. At the heart of the genetic determination of lifespan is the extent to which the organism's genome invests in survival. Because life is usually 'nasty, brutish and short', it serves no purpose to squander resources on maintenance and repair in an attempt to last indefinitely — this idea that the body must be expendable is the core of the 'disposable soma' theory[5,9].

But if ageing is a matter of things falling apart, can research realistically hope to achieve anything useful? The answer is emphatically yes — there is plenty of evidence that it is possible to intervene in the underlying causative mechanisms. Indeed, the malleability of the ageing process, as revealed by demography, derives precisely from the fact that it seems to be possible to slow the rate at which damage accumulates. Human longevity continues to increase when further gains from reducing mortality earlier in life are negligible because nowadays we reach old age, on average, in better condition than ever before.

Our understanding of the ageing process has advanced to a point at which it can be summarized relatively simply: molecular and cellular damage eventually results in frailty and disease (Fig. 2). But the devil is in the details. A great deal of complex biological research is needed to understand precisely which factors underlie our increasing longevity, and how far 'healthy ageing' is attainable. Even if, as some fear, obesity and sedentary lifestyles combine in the future to slow, or even reverse, the increase in life expectancy, we still need this understanding because unhealthy living usually drives people to early graves through pathology that is, to a significant degree, age-related.

## Can't beat the systems

It was recently suggested that 'robustness' is one of the fundamental characteristics of biological systems, and that building a solid theoretical foundation of biological robustness is a key challenge for systems biology[10]. This is unquestionably true, but so is the converse: it is the pervasive vulnerability of living systems to damage, which erodes the functionality of the mechanisms underpinning robustness, that lies at the heart of ageing and disease. If the general tendency of natural selection has been towards greater robustness, then understanding ageing is the way to secure insights into the limitations and trade-offs that make robustness imperfect.

An enormous range of faults arises regularly in molecules, cells and tissues (Box 2). For each of these there is evidence that the relevant lesions do indeed accumulate during ageing, but for none of them do the faults seem sufficiently numerous to cause the systematic deterioration and loss of function that characterizes the senescent phenotype. Furthermore, because nearly all the mechanisms are intrinsically stochastic (subject to the laws of chance), there is marked variability from molecule to molecule, cell to cell, and individual to individual[11]. It is this combination of multiplicity and stochasticity of mechanisms that means there can be little expectation of a satisfactory understanding of ageing without adopting an integrative approach for exploring the synergy and interactions of the different mechanisms.

The need for a systems understanding is shown by the large volume of data on the possible contribution of oxidative damage to the mechanisms that underpin ageing. The suggestion that reactive oxygen species (commonly known as free radicals) cause ageing is already half-a-century old, and there is plenty of evidence to support this idea. Comparative studies have shown a strong association between the longevity of a species and the capacity of its cells to withstand oxidative stress[12]. But how

**Box 1 | What is systems biology?**

Systems biology can be viewed in a number of ways, as follows:

1. As a discipline or **field of study** in its own right, involving the quantitative analysis of interactions between elements of biological systems. There is an emphasis on complexity and large data sets, which are typically produced by a variety of high-throughput genomic, proteomic and metabolomic techniques.

2. As a set of multidisciplinary **methodologies**, in which the emphasis is placed on cycles of iteration between experimental data collection and computational or mathematical modelling. These lead to further development of theory, which in turn motivates new experimental investigations.

3. As an **integrative approach**, offering an alternative to the 'reductionist' approach that is seen by many to have dominated the research agenda for years.

4. As an **organizational phenomenon** involving the bringing together, in exceptionally close working partnerships, of scientists from diverse disciplinary backgrounds, particularly the biological, engineering and mathematical sciences.

Most initiatives in systems biology include several of these features. **T.B.L.K.**

**Box 2 | Damage that may contribute to ageing**

**DNA damage (genome instability)**
- Somatic mutations (copying errors, imperfect repair)
- Telomere shortening
- Chromosome rearrangements
- Mitochondrial-DNA mutations
- Gene disruption by viruses, transposons etc
- Aberrant epigenetic modifications

**RNA damage**
- Transcription errors
- Aberrant splicing

**Protein damage**
- Misfolding
- Synthesis errors
- Aberrant post-translational modifications
- Aberrant aggregation
- Impaired protein turnover (catabolism)

**Membrane damage**
- Oxidation

Additionally, there may also be disruption through stochastic variation in gene expression, cell-fate determination, differentiation, damage segregation during cell division, cell migration and cell death. **T.B.L.K.**

do we account for the fact that some long-lived species, notably naked mole rats, have exceptionally high levels of oxidative damage[13]; that transgenic disruption of key components of antioxidant defences does not necessarily affect lifespan[14]; and that dietary supplementation with antioxidants seems to have little or no effect? It seems a fair bet that answering these questions will require a much more systematic look at the complex network of reactions through which reactive oxygen species are generated and by which the cell defends itself.

## Metabolic regulation

One of the most exciting areas of progress in ageing research is the discovery of metabolic factors that influence longevity. These include genes that affect insulin-signalling pathways[15], such as *daf-2* in *C. elegans*, the action of proteins known as sirtuins[16], or externally imposed changes in food supply (dietary restriction). In each case, the route to altered longevity seems to have some impact on the way the cell's maintenance systems are controlled.

Given the centrality of resource allocation in understanding how trade-offs are mediated between maintenance, growth and reproduction, it is not surprising that metabolic factors can modulate the level of maintenance. The scope of such effects merits study from an evolutionary perspective. In the case of *C. elegans*, an evolved metabolic plasticity is evident in the alternative developmental pathway that, when nutrients are scarce, generates the long-lived 'dauer' larva, a stress-resistant dispersal form[17]. This innate plasticity provides the basis for several of the most dramatic life-extension mutants in this species. In mice, quantitative modelling of the evolutionary energetics of reproduction and maintenance suggests that the life-extending effects of dietary restriction might be adaptive — as a means to wait out times of famine — but only under certain conditions[18]. The crux of the matter is whether enough energy can be diverted from reproduction to maintenance to make a physiologically important difference and whether, in a world where mortality pressure is high anyway, it actually boosts darwinian fitness. For humans, in whom reproduction is proportionately much less costly than in mice, the same logic suggests that dietary restriction is unlikely to postpone ageing[19].

Despite the evidence that metabolic regulation can have large effects on longevity, at least in small animals, it is important to note that it affects principally the rate of ageing and not, apparently, its nature. This preservation of the intrinsic complexity of the underlying molecular and cellular pathology is strikingly revealed in *C. elegans*, for example, where there is a large degree of stochastic variation within any given experimental regimen[20–22]. This provides a deeper challenge for the systems biology of ageing: to deliver the potential to intervene in the ageing process in ways that can specifically enhance the quality of later life. It seems highly unlikely that the ageing process itself will be abolished any time soon so, even if metabolic interventions are found to extend the years of healthy life in humans, we still need to grapple with the problem of age-related disease.

## Ageing and disease

Age is by far the biggest risk factor for a wide range of clinical conditions that are prevalent today. One might therefore presume that a major effort is being made to understand the ways in which ageing renders the elderly more vulnerable to pathology. Nothing could be further from the truth. There is a large number of medical research institutes around the world, many with a focus on one or more of the major age-related diseases — cancer, heart disease, arthritis or dementia. Yet only a tiny fraction of these carries out any research on the intrinsic contribution from the ageing process itself.

Given that ageing is driven by damage, and that this is also true for the many age-related diseases, there must be considerable overlap between the underlying causative pathways. In cases of 'normal' brain ageing, for example, in which an older person's cognition remains essentially intact, autopsies reveal almost as many neurofibrillary tangles and amyloid plaques as are seen in patients with Alzheimer's disease[23]. This suggests that these lesions, which are taken as diagnostic for Alzheimer's, are far more closely connected with intrinsic ageing than is commonly thought, prompting questions about the role of underlying systems properties of the ageing brain.

Common factors, such as oxidative stress, are implicated in several age-associated diseases and, as in ageing itself, there may be important synergy between mechanisms. For instance, an accumulation of dysfunctional mitochondria will give rise to a decline in energy production, which in turn will lead to a decline in the efficacy of cell maintenance systems such as protein turnover, resulting in pathogenic protein aggregates, and so on. This may explain the association between mutations in mitochondrial DNA and neuronal death in the substantia nigra of brains in patients with Parkinson's disease[24,25].

One set of disorders in which the connections with ageing are particularly striking are the inherited human 'progeroid' syndromes, such as Werner's syndrome, in which the mutation of a gene coding for a DNA-maintenance enzyme causes early onset of multiple pathology. Such conditions, and their corresponding models in the mouse, highlight the complexity that needs to be understood. For example, in mice with a mutation in the *Ercc1* gene, increased DNA damage was recently discovered to cause the altered expression of more than a thousand genes, including downregulation of metabolic factors and upregulation of antioxidant and DNA repair pathways[26]. Although at first sight it seems surprising that the pattern of altered gene expression in this short-lived mutant was the same as that reported in long-lived genetic mutants or diet-restricted mice, it is entirely plausible from a systems perspective that widespread damage should trigger pathways that invoke heightened protection against such damage. Thus, the cycles of cause and effect are complex and can have different origins.

The connection between ageing and disease is profoundly important in cancer. Total cancer

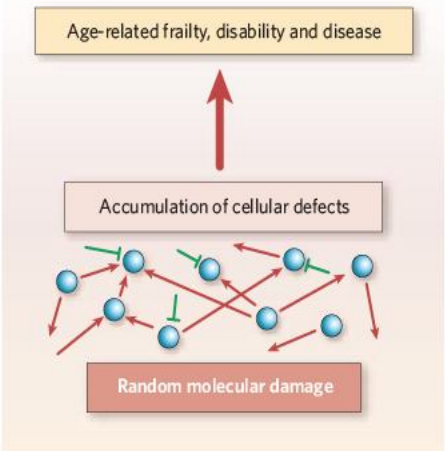


**Figure 2 | The mechanisms of ageing.** The ageing process is driven by the lifelong accumulation of molecular damage, leading to age-related frailty, disability and disease, and eventually to death. Although individual instances of damage are essentially random, the overall rate at which damage accumulates is regulated by a complex array of maintenance and repair pathways, which in turn may be modulated by metabolic factors. This scheme readily accommodates genetic influences on longevity (via the setting of maintenance functions) as well as contributions from environmental and lifestyle factors, which can influence exposure to damage and the capacity to withstand it.

**Box 3 | Case study: systems biology of cellular senescence**

An extensively studied model of cellular ageing is the cultured human diploid fibroblast, which divides only a finite number of times before entering a state of 'replicative senescence' (this number is known as the Hayflick limit). Although senescence is commonly attributed to simple telomere erosion, there is remarkable cell-to-cell heterogeneity in division potential.

There is evidence that the rate of telomere shortening is strongly affected by oxidative stress, and that an important source of damage-inducing 'free radicals' (reactive oxygen species) is the intracellular population of mitochondria, particularly those that are themselves damaged by random mutation. As a result, a mathematical model was developed that showed how the heterogeneity of cell senescence can be explained by the synergy of multiple mechanisms (oxidative damage, telomere shortening and the stochastic nature of mutation to mitochondrial and nuclear DNA)[31].

These modelling predictions prompted the experimental study of a role for mitochondrial dysfunction in senescence[32], something previously unexplored. Confirmation of this role has opened a new vista on the complex interactions underlying ageing in dividing cells. It has also provided a wealth of fresh and challenging data that will re-enforce the development of more detailed and realistic models, driving the process of discovery through a cyclic interaction between modelling and data. **T.B.L.K.**

incidence rises steeply with age and, across species, scales with longevity. The core of the connection is, of course, damage — how cells guard against it, and how they respond to it when it arises. Long-lived species invest in better maintenance, which delays both ageing and cancer. On the other hand, when damage does occur, it seems that cancer and ageing sit on opposite sides of a see-saw[27]. Deleting damaged cells too readily can protect against cancer but accelerates other forms of age-related pathology that are linked to cell loss. As we continue to discover an ever-increasing variety of types of damage-induced cellular senescence and probe their connections with cancer[28], we will need to build this knowledge into a systems framework.

## Implementing systems approaches

Some substantive commitment will be needed to realize the huge potential benefits of addressing the challenge of ageing from a systems perspective. As in systems biology generally, scientists from biology, bioinformatics, computing science, mathematics, statistics and engineering must be enabled to build enduring partnerships. We have seen the creation of some systems-biology institutes, which provide physical co-location, but, although they are useful as exemplars, it is by no means obvious that in the longer term such ventures will be necessary or even optimal. Systems approaches need ultimately to be incorporated into the working practices of a majority of scientists.

Designing experiments that can simultaneously combine the contributions of different mechanisms to the ageing process is not easy (see Box 3). However, provided that experiments are planned with a view to making firm connections with other data, it is possible to structure an accumulation of knowledge in integrative models and in well-curated data archives[29]. For example, the Biology of Ageing e-Science Integration and Simulation (BASIS) system[30] provides a facility, supported by web services, for building, synthesizing and simulating mechanistic models using the Systems Biology Markup Language (SBML). An excellent example of a large-scale project in integrative systems biology is the Human Physiome Project. As further examples accrue, the momentum is expected to build.

The advances of recent years in understanding the mysteries of ageing are spectacular, but in truth we have only scratched the surface of this extraordinarily difficult problem. The intrinsic nature of the ageing process is essentially one of systems degradation. Only by systematically probing the complex mechanisms underlying ageing and its associated diseases can we transform our dramatic past successes in postponing death into a future in which ageing will, hopefully, lose some of its sting. ■

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# Chemistry for everyone

Peter Murray-Rust

**Moves by chemists to help computers access the scientific literature have boosted the drive to make scientific information freely available to all.**


Imagine a world in which all scientific information is instantly available — where anyone can get an answer to any question, no matter how abstruse or dependent on technical formalism. In such a world, science is published directly onto the Internet, the size of data sets doesn't matter, and machines can do dirty and everyday tasks, such as searching through millions of dense technical articles or calculating routine data. This is the emerging world of e-science or cyberscholarship.

e-Science seeks to develop the tools, content and social attitudes to support multidisciplinary, collaborative science. Its immediate aims are to find ways of sharing information in a form that is appropriate to all readers. This requires new methods for gathering and representing data, for improved computational support, and for growth of the online community. But who are the readers? They are not only professional scientists, but also children, senior citizens, lawmakers and funding bodies. And they are not only people — information must be accessible to machines, because humans won't be able to cope with the amount and complexity of the incoming data.

Cyberscholarship also embraces the revolution of 'web 2.0', in which humans and machines come together in unpredictable ways to create innovative knowledge resources. The resulting 'cyberlaboratory' — foreseen in the novels of William Gibson and others — is giving rise to virtual spaces where scientists share ideas and data, and where some of the traditional values of science are re-examined in emerging 'gift economies'.

Here I will show how chemistry, often thought of as a conservative discipline, is making important contributions to the nascent field of e-science. Indeed, the creativity shown by young chemists might transform the way that science is performed.

## Open information

In the twentieth century, technical information was expensive, as it was gathered by humans, double-checked and then usually retyped. For example, the American Chemical Society collects bibliographic information and abstracts for all chemistry-related articles published worldwide. Its Chemical Abstracts Service (CAS) contains data on more than 27 million substances and is seen by chemists as the fundamental source of chemical information. But in the twenty-first century, this resource — along with all the other conventional sources of chemical information — is incompatible with the requirements of web 2.0. If chemists are to contribute to e-science, they must rethink their approach to the way information is accessed and presented.


**Figure 1 | Virtual world.** Second Life is a virtual world that allows people to interact using animated characters, or avatars. Here, people are discussing open science. Inset, an interactive molecule.

Even so, chemists — with almost no funding — have created some of the best aspects of web 2.0. The Nature Publishing Group has watched these developments carefully and encouraged them by providing scientific commentary blogs, discussion forums in the virtual-reality world of Second Life (Fig. 1), and, more recently, by co-sponsoring 'Foo camps'. These interdisciplinary brainstorming meetings exploit web 2.0 ideas to full advantage, and have helped to legitimize and encourage unconventional approaches to sharing information. Such fun initiatives may look trivial and are currently inefficient. But they emphasize the power of collaboration and show how verbal communication can be enhanced online. They are a serious part of the future of science.

But there is more to be done. A group of chemists, programmers and informaticians — myself included — have set up an informal, online community known as the Blue Obelisk to encourage openness in chemistry. The mantra is "open data, open source and open standards". The Blue Obelisk is a bottom-up movement, largely composed of young researchers inspired by web 2.0 and by the relative ease with which useful chemical software can be written. The emphasis is on open, interoperable software, reference data and algorithms, such as Jmol, which allows computer models of molecules to be displayed in various ways, and Open Babel, which interconverts the different electronic formats used to store chemical information. The Blue Obelisk provides a complete basic infrastructure for open-access chemistry, including a standard language for communication (chemical markup language, CML) and libraries of software applications for essential chemical functions (the Chemistry Development Kit, CDK).

The issue of open data is particularly problematic. Unlike astronomers, geoscientists and biologists, chemists have no global data-collection projects; their data are usually published in many different online journals and then collated by hand into CAS. In the era of real

paper, limited page counts ensured that most chemical data were never published, and so are effectively lost. Even now, most electronic documents still use visual representations of a printed page (such as PDF files), rather than machine-friendly formats that allow data to be shared across different information systems. Moreover, the default business model for chemical publishing is 'reader pays'. As a result, non-subscribers — that's most of the world — have no access to a large percentage of chemical data.

But things are changing. The web is an almost infinite, comprehensive source of free data. Young scientists don't go to libraries and no longer look to traditional sources of information, but to search engines such as Google. They expect to be able to express their questions in natural language and to get instant answers. They have no time to learn proprietary systems with idiosyncratic approaches. For reference, one of the first places to look is Wikipedia.

Although relatively few chemists contribute to Wikipedia, the quality of its chemical content is high and increasing. Chemistry is an ideal subject for recording as factual information, and Wikipedia will soon be acknowledged as the primary reference for chemistry undergraduates. Proactivity is the key — if you find errors, correct them. And through the use of 'infoboxes' that contain searchable data, Wikipedia will evolve into a computer-searchable reference source that is more advanced than those provided by conventional commercial suppliers.

But many of the problems associated with capturing data are not technological but social. Most research institutions undervalue pure data, focusing instead on published papers as the hallmark of academic achievement. This is exacerbated by publishers, who generally do not require — and often oppose — the mounting of open data sets. Only 0.1% of the analytical spectra for the 20 million or so published compounds are openly available. But e-science and the demands of global problems are forcing this situation to change; data journals are starting to appear and will create markets for high-quality, citable data.

To encourage open data, my research group and others are exploring several ways of capturing data at almost zero cost. In the SPECTRa project, spectroscopic and crystallographic data are sent directly to open repositories. Again, the main barrier is social: many scientists wish to hide their data to prevent others from using them to their own advantage, showing them to be fallacious or making them unpatentable. Unfortunately, this leads to rapid data loss (often 80–99%). To avoid this, SPECTRa has surveyed how chemists actually work and proposes an 'embargo repository' that allows data to be released only after an appropriate period.

Another option is to make data publication a condition for all papers. For example, the International Union of Crystallography (IUCr) has campaigned over many years for the publication of raw crystallographic data and metadata (data about the data). As a result, more than 30% of all published crystallography data are openly available. CrystalEye is a web-based system that exploits this openness by scouring the Internet for crystallographic data and collecting them together in a searchable format (Fig. 2). Currently, CrystalEye has more than 100,000 entries gathered from daily visits to online journals. It uses Blue Obelisk software, such as Jmol and Open Babel, and the data are freely available for all users to use, reuse and redistribute.

The third strategy for cheap data capture is to extract data electronically from existing text. But there are problems with this. Text is usually only meaningful to people — there are few semantic flags that would allow machines to understand it. Furthermore, the full text of many documents is often not open access, and even if it were, many authors and publishers will not allow data to be extracted robotically from their work. Nevertheless, good progress has been made with natural-language processing software. For example, the OSCAR3 program, developed by my group, investigates how chemical information can be extracted from the text in PDF files.

But the single most crucial thing that chemists should do to simplify data capture is to abandon paper and create digital-only, semantic documents that can be understood by computers.

## Metadata, semantics and ontology

The graph shown in Figure 3 (overleaf) is a good example of an object that is semantically void to a computer — humans can extract much meaning from it, but machines can understand nothing. To make it useful for e-science, we must represent the data as numbers in a standard form; use metadata to label the axes; and interpret the chemistry by mapping 'carbon dioxide' to a standard definition, such as that found in the open-access PubChem database (which lists more than 10 million compounds, each with its own universally agreed identifier). Adding such details to chemistry documents is simple and costs nothing, but is essential to allow a free flow of information.

It is more difficult to add ontological information — metadata that define concepts — to text. Figure 4 shows part of a paper in which chemical terms are recognized by the OSCAR3 language-recognition software. Ontological markers are used, so, for example, the word 'desilylation' is recognized as a chemical reaction, and the Greek letter 'α' is identified as a chemical prefix. Chemical names are also recognized; these can be linked to structural information in molecular databases (such as PubChem, ChEBI or the Gold Book), from which chemical structures can be produced and manipulated using open software such as CDK. The Royal Society of Chemistry uses this approach to enhance online publications in its award-winning Project Prospect, which builds in part on a collaboration with my group.

Creating ontologies is laborious, so it is useful if the load can be spread. For example, the

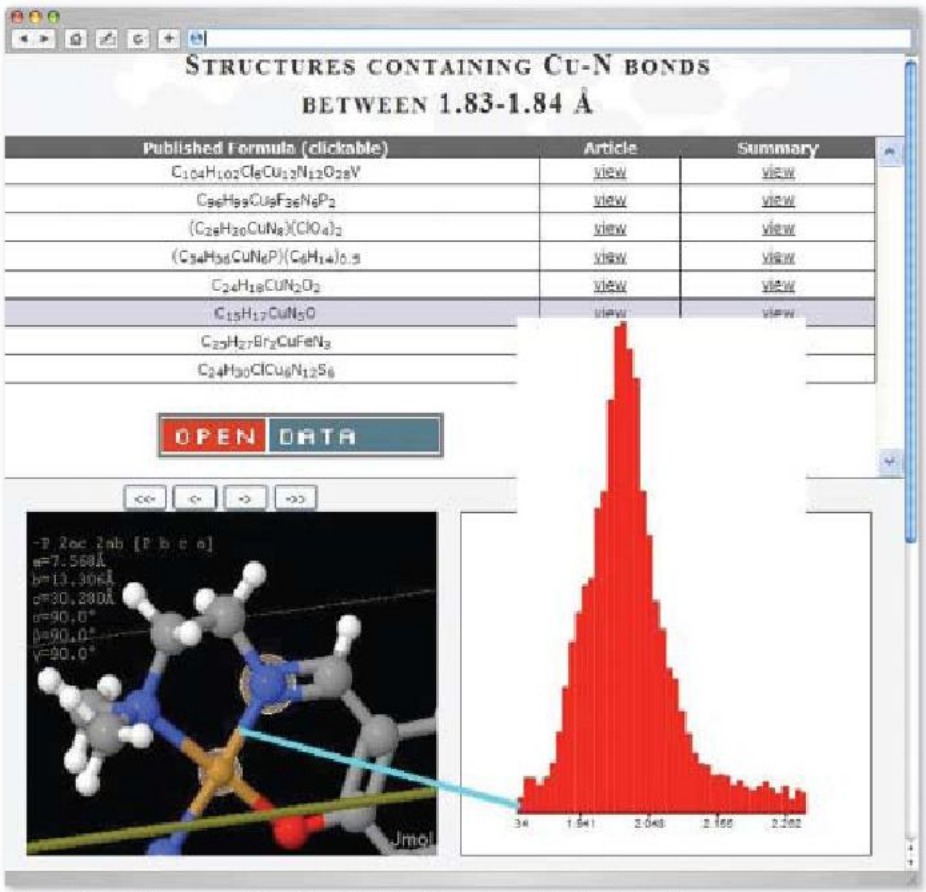


**Figure 2 | Exploiting open data.** CrystalEye is a free web application that gathers open-access crystallographic data and allows it to be searched and manipulated. The screenshot shows the results of a search for compounds with copper–nitrogen bonds. The graph plots the number of hits against the lengths of the bonds; compounds with the shortest bonds are listed in the table. The molecular structure of the compound highlighted in the table is also displayed.

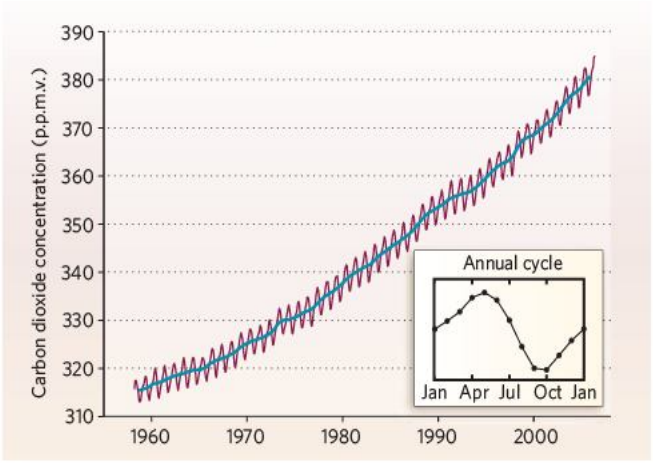


**Figure 3 | Meaningless data?** A Keeling curve plots the concentration of carbon dioxide in the atmosphere above Mauna Loa, Hawaii, since 1958. Although humans can obtain much information from this graph, it is meaningless to computers unless identifiers are added that allow the data to be interpreted electronically.

IUCr's crystallographic information file (CIF) system is the result of a 15-year collaborative effort. This universal text-file format allows many different computer programs to interpret crystallographic data in order to share and visualize molecular structures.

Ontology can also be added to numeric data. Similarly, computer code can be converted into a 'universal' language — for example, several of the codes often used for quantum mechanics, molecular mechanics and crystallographic calculations have been converted into CML. The semi-structured CML vocabulary can then be reformatted in different ways to allow different programs to use the codes, using cross-platform toolkits such as FoX software (which allows FORTRAN programs to produce output in modern formats such as CML or XML).

To extract structure and relationships from heterogeneously computed data, software such as Golem has been developed in our group. Golem spots patterns that describe how data are expressed in chemistry documents (whether written by humans or machines), and uses these patterns to extract and correlate data from document repositories. This could reveal meaning that was not explicit in the original versions.

Ontologies are powerful when dealing with large amounts of text and data, as they can exploit 'triple' relationships. Triples are statements that come in three parts: subject, predicate and object (the predicate defines the characteristics of the subject, and expresses a relationship between the subject and the object). A simple example is: "The car has the colour red." Here, the car is the subject and the predicate "has the colour" describes the relationship of the car to the object, which is red. Triples can describe almost any concept and can be described in standard formats that are recognized by machines. For example:

pubchem:CID280 pubchem:name "carbon dioxide"

Translated into human terms, this means "the chemical defined as CID280 in the PubChem database has the name carbon dioxide". Triples can each be given a unique location (similar to a URL on the web) and saved in triple stores. If semantic flags in electronic documents link to triples, then any computer can extract information from those documents. Used in combination with each other, triples allow machines to deduce a deeper 'understanding' of information. Several organizations and companies espouse this vision of the semantic web, and are building stores that can host gigatriples of information.

This approach for data mining has come of age, as highlighted by the DBpedia project, which extracts triples from the categories and infoboxes in Wikipedia. This allows sophisticated questions to be asked of Wikipedia, by linking together information spread across the entire resource. Remarkably, DBpedia arose spontaneously — no authority orchestrated it, and the volunteers who wrote entries for Wikipedia had no idea that their work would be used in this way. Although DBpedia is currently poor at extracting chemistry information, its potential can be shown by real queries such as:

Soccer player [...] number 11 from club with stadium with >40,000 seats born in a country with >10 million inhabitants

The question simply links five triples, but amazingly it returns a short, precise list of names fulfilling the criteria. We have now created triples for more than 1,000 chemical compounds in Wikipedia and shown that similar queries can be used to mine these data. This search method will be ideal for finding chemistry information once the appropriate ontologies are created.

## Social computing and collaboration

This decade has seen an explosion in social computing, in which humans and information systems have become greatly interconnected. Such connectivity is essential to meet the needs of e-science. The most valuable elements of the social computing 'ecosystem' include wikis, blogs, virtual collaborative environments and recommender systems, which suggest links of interest to readers based on their previous choices, creating a meritocracy of information. Unfortunately, all these innovations are currently limited to text and images, and lack interfaces for adding scientific material such as equations, chemistry, molecular visualization or computer code. There is a pressing need for a standard system of scientific tools in this area, including plug-ins for browsers.

Even so, the chemical 'blogosphere' has been spectacularly successful. At least 100 bloggers produce consistently interesting content, ranging from laboratory chat to accounts of actual experiments with photographs, gels and spectra. Some blogs are personal review journals, with commentaries on chemical articles; others report on drug discovery, patents and business. There is even a 'meta-blog' that reviews the other chemistry blogs, and which has pioneered semantic links for the automatic extraction of chemistry information from these resources.

A crucial group of technology blogs focuses on software and data, most of which is open access. One development is Blue Obelisk's 'greasemonkey', a browser plug-in that alerts readers to unseen features in the pages they are viewing. It can highlight any publication mentioned in the blogosphere, or any paper that has a structure in CrystalEye. This provides an alternative mechanism for reviewing the literature, and allows chemists to assess the quality of papers, independent of impact factors. None of this requires consent from publishers.

There are many new approaches to social computing and data sharing, of which the dynamic, interactive world of Second Life is well known. The Blue Obelisk community has invested in some virtual real estate and collaborated with *Nature* in a new generation of interactive arenas and intelligent objects. Encouraged by iPhones and multi-touch screens, we expect the next few years to revolutionize the way that humans interact with information. This will inevitably find its way into everyday scientific practice.

Another critical aspect of collaborative science is the open notebook, which records experiments on the Internet as they happen. When coupled with semantic documents, this generates globally visible, machine-readable information. It challenges the current ethos that chemists may not disclose their work before it has been formally published. Open notebooks are especially suited to computational and simulation processes.

My group, in collaboration with researchers at Imperial College, London, has recently mounted a system that routinely predicts analytical spectra for new compounds presented in publications. Such a system could act as a robot reviewer to judge whether published data seem reasonable. Predicted spectra would be published as soon as they are calculated, so the whole world can comment on the method and individual data (both for the experimental data and the predictive software). A publisher could then be approached to give the final seal of approval to experimental data.

## Processing power and data storage

Currently, most of the information typically published for an organic compound can be stored using just 1 megabyte of data. With about 1 million new compounds discovered every year, this amounts to a paltry annual output of just 1 terabyte — less than a single day's calculation output for some astronomical or geophysical laboratories.

Clearly there are gaps in chemistry data. As an example, high-quality molecular modelling data are not available for most compounds, although in the majority of cases such information can be calculated in just one or two days. The process of modelling chemical structures is well suited to high-throughput, simultaneous computation. Only about 5,000 machines would be needed to calculate fundamental data for the world's annual complement of new compounds. Several groups, including my own, have therefore taken over spare computer capacity — such as university teaching machines at night — to fill this data gap.

Of course, such initiatives create problems of data storage. Fortunately, many companies are now supporting open systems and data — at the 2007 Science Foo Camp, for example, Google offered to host open scientific data free of charge. In the emerging arena of community systems and content, data and software will be free, and openness will be seen as a big commercial advantage.

## Simple technology and decentralization

The web is not just a triumph of technology — it is equally as dependent on human input. If the Internet is to develop successfully, systems are needed to make human involvement as easy as possible. The current basic protocols for web interactions (such as SOAP) are heavily engineered approaches that are unnecessarily complex, so many information technologists are adopting a new style of software architecture known as REST, which is much simpler.

Most REST applications are based on features in the hypertext transfer protocol (HTTP, the standard rules used to transfer information on the web), and the uniform resource identifier (URI) framework, which gives all information an 'address' in a common format. A key advantage is that the interfaces to REST applications are simple and uniform, whereas older systems often required specific implementations and tools to allow different software to 'talk' to each other. As a result, REST allows users to focus on their data, rather than having to second-guess how any particular set of users might want to use it. Frameworks and tools that support REST tend to embrace this simplicity throughout their design — REST systems are easier and quicker to use. For scientific data, the combination of triples with REST is replacing traditional portal services.

The next few years will see a shifting balance between data and computation held locally and centrally. Decentralization is often the key to high-throughput data processing, for example by farming out tasks to any idling machines in a network. A crucial strategy is seen in the MapReduce system pioneered by Google. In this approach, users send data sets to a hub, which distributes the data across hundreds of thousands of computers for processing. After reduction of the outputs, the results are returned to the user. But because the service relies on a central provider, there is a danger that openness could be destroyed. The traditional approach, in which chemists store and process their data locally, will still have value.

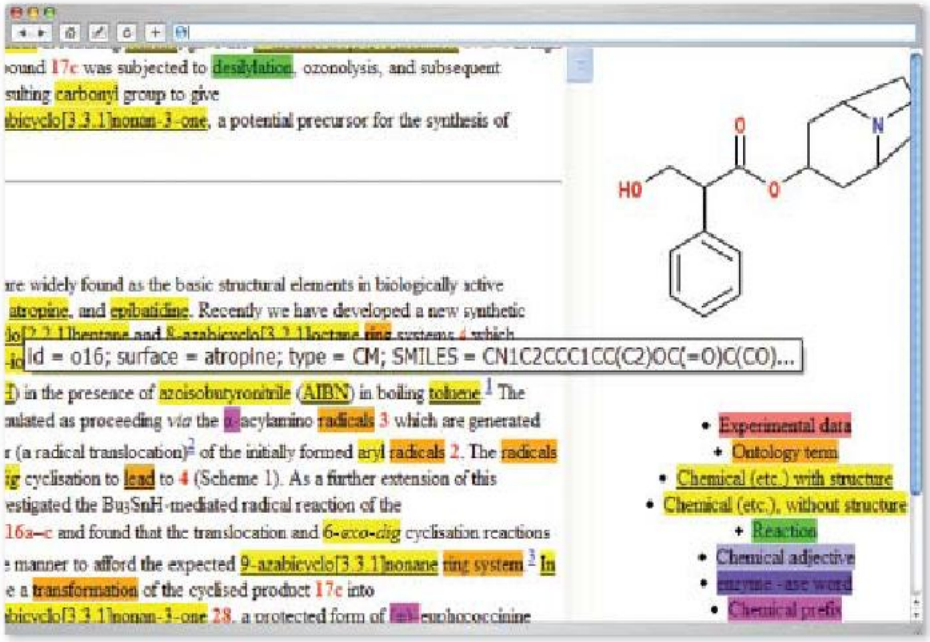


**Figure 4 | Text for computers.** OSCAR3 is a free web application that can extract chemical information from text in natural language. In this screenshot, the highlighted text has been recognized and categorized according to the colour key (bottom right). By selecting the word 'atropine' in the text, structural information for the compound is retrieved from the web and converted into a manipulable structure (top right) by another open-source algorithm.

A concern for scientists is that web 2.0 is based on human vocabulary, which can be ambiguous. For example, chemists would recognize 'CO' as the chemical formula for carbon monoxide, but a computer would confuse it with the abbreviation for Colorado; browsers currently rely on humans to distinguish between such things. The Blue Obelisk has therefore developed a browser with chemical 'intelligence', known as Bioclipse, based on an open-source development framework known as Eclipse. Bioclipse can recognize molecules, reactions, proteins and their sequences, spectra and crystallographic data, and fire up specific applications to handle each of them. When semantically rich data become common on the web, and discipline-specific search engines evolve, cyberscience will truly have arrived.

## Conclusions

The world is changing rapidly, and the chemistry establishment must adapt quickly or fracture. Closed publications, binary software and toll-access databases are being swept away by the emerging philosophies and technologies. Many young scientists do not read or use closed systems, and are increasingly frustrated by out-of-date approaches. Perhaps for the first time in history, the technology for change is in their hands — indeed, several of Blue Obelisk's systems were pioneered by undergraduates. As new ideas and technologies arise, the blogosphere spreads them almost instantaneously. And the message from the blogosphere is clear: the next generation of chemists needs open, integrated, semantic systems.

If chemical information is to address worldwide problems, it must be made open as rapidly as possible. This will involve working alongside the publishers that currently produce most of the scientific literature. But we also need new social protocols, as the current ones aren't working. So here are some suggestions, based on the spirit of the blogosphere. We must support young people; they are already shaping the future through interactive collaborative systems. We must use global challenges — such as climate change, disease and the ageing population — as spurs to drive the evolution of our information systems. And we should reach out to unconventional communities for their ideas.

But perhaps most importantly, the information economy must be redesigned so that rewards are given for making information open. If we can create a US$30-billion carbon-trading market to help deal with carbon dioxide emissions, why can't we sell chemical-information credits, rather than journal subscriptions? This would require government action, but it could be made to work. There is broad support for such a move. But you don't have to take my word for it. Ask the blogosphere. ■

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FURTHER READING AND ONLINE RESOURCES

Hundreds of people have contributed to open chemistry and they are best acknowledged by following the links from the following web pages. Most projects also have Wikipedia entries.

- http://wwmm.ch.cam.ac.uk
- http://blueobelisk.sourceforge.net/wiki/index.php/Main_Page
- http://cb.openmolecules.net
- http://www.okfn.org
- http://en.wikipedia.org/wiki/List_of_chemistry_topics

# Building better batteries

M. Armand and J.-M. Tarascon

**Researchers must find a sustainable way of providing the power our modern lifestyles demand.**

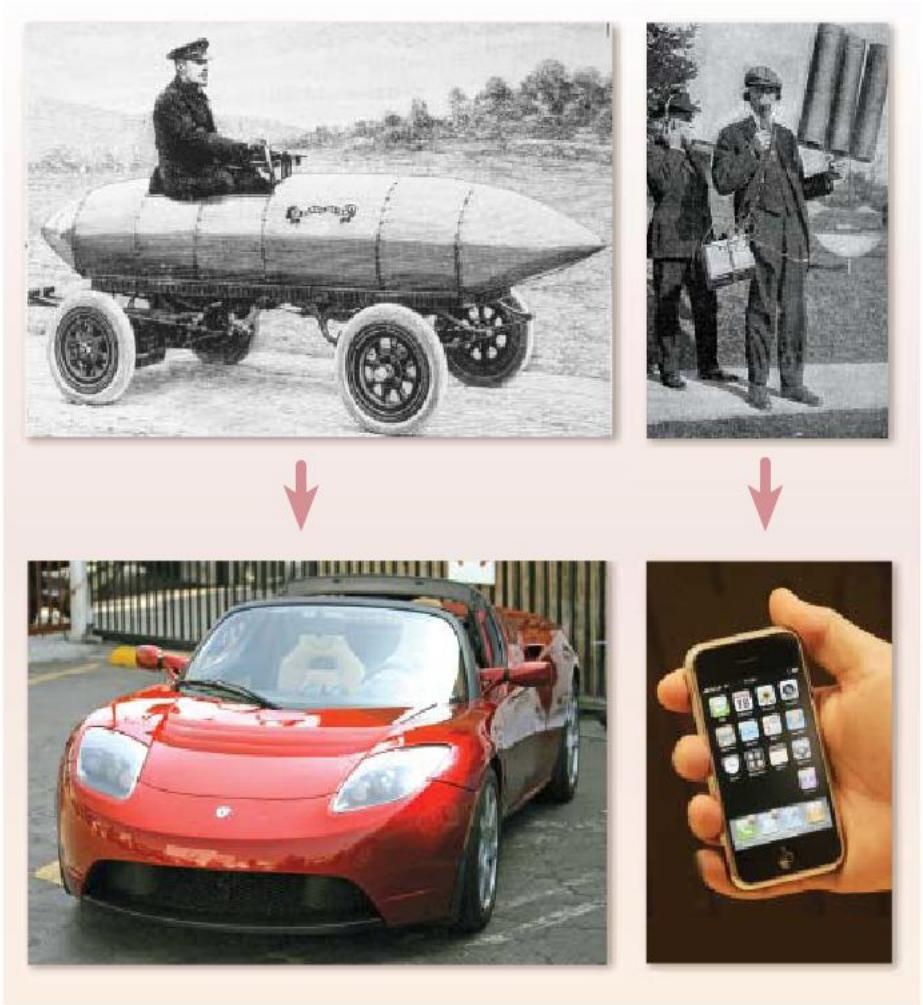

Batteries are currently being developed to power an increasingly diverse range of applications, from cars to microchips. How can scientists achieve the performance that each application demands? How will batteries be able to power the many other portable devices that will no doubt be developed in the coming years? And how can batteries become a sustainable technology for the future?

The technological revolution of the past few centuries has been fuelled mainly by variations of the combustion reaction, the fire that marked the dawn of humanity. But this has come at a price: the resulting emissions of carbon dioxide have driven global climate change. For the sake of future generations, we urgently need to reconsider how we use energy in everything from barbecues to jet aeroplanes and power stations.

If a new energy economy is to emerge, it must be based on a cheap and sustainable energy supply. One of the most flagrantly wasteful activities is travel, and here battery devices can potentially provide a solution, especially as they can be used to store energy from sustainable sources such as the wind and solar power.

Because batteries are inherently simple in concept, it is surprising that their development has progressed much more slowly than other areas of electronics. As a result, they are often seen as being the heaviest, costliest and least-green components of any electronic device. It was the lack of good batteries that slowed down the deployment of electric cars and wireless communication, which date from at least 1899 and 1920, respectively (Fig. 1). The slow progress is due to the lack of suitable electrode materials and electrolytes, together with difficulties in mastering the interfaces between them.

All batteries are composed of two electrodes connected by an ionically conductive material called an electrolyte. The two electrodes have different chemical potentials, dictated by the chemistry that occurs at each. When these electrodes are connected by means of an external device, electrons spontaneously flow from the more negative to the more positive potential. Ions are transported through the electrolyte,



CCI ARCHIVES/SPL

S.CURRY/AFP/GETTY

G. RUELAS/REUTERS

**Figure 1 | Revisiting the past.** In 1899 a Belgian car, *La jamais contente* (top left), equipped with lead–acid batteries, reached a speed of 30 metres per second (ref. 26). In the same year, at a car competition in Paris, the only petrol-driven car was disqualified for having unpractically high consumption. Inside the United States, between 1900 and 1920, the proportion of electrical cars produced fell from 60% to 4% of the total. One century later, fully electrical cars, such as the Tesla roadster (bottom left), are coming back into the picture. Meanwhile, the first wireless communication took place in Pennsylvania in 1920 (top right, after ref. 27). Nearly 100 years later, the latest mobile phones (bottom right) can perform a wide range of functions.

maintaining the charge balance, and electrical energy can be tapped by the external circuit. In secondary, or rechargeable, batteries, a larger voltage applied in the opposite direction can cause the battery to recharge.

The amount of electrical energy per mass or volume that a battery can deliver is a function of the cell's voltage and capacity, which are dependent on the chemistry of the system. Another important parameter is power, which depends partly on the battery's engineering but crucially on the chemicals the battery contains. Hundreds of electrochemical couples were proposed during the nineteenth and early twentieth centuries, the most notable primary battery being Zn–$MnO_2$, with lead–acid and Ni–Cd being the most common secondaries[1].

The stored energy content of a battery can be maximized in three ways: (1) by having a large chemical potential difference between the two electrodes; (2) by making the mass (or

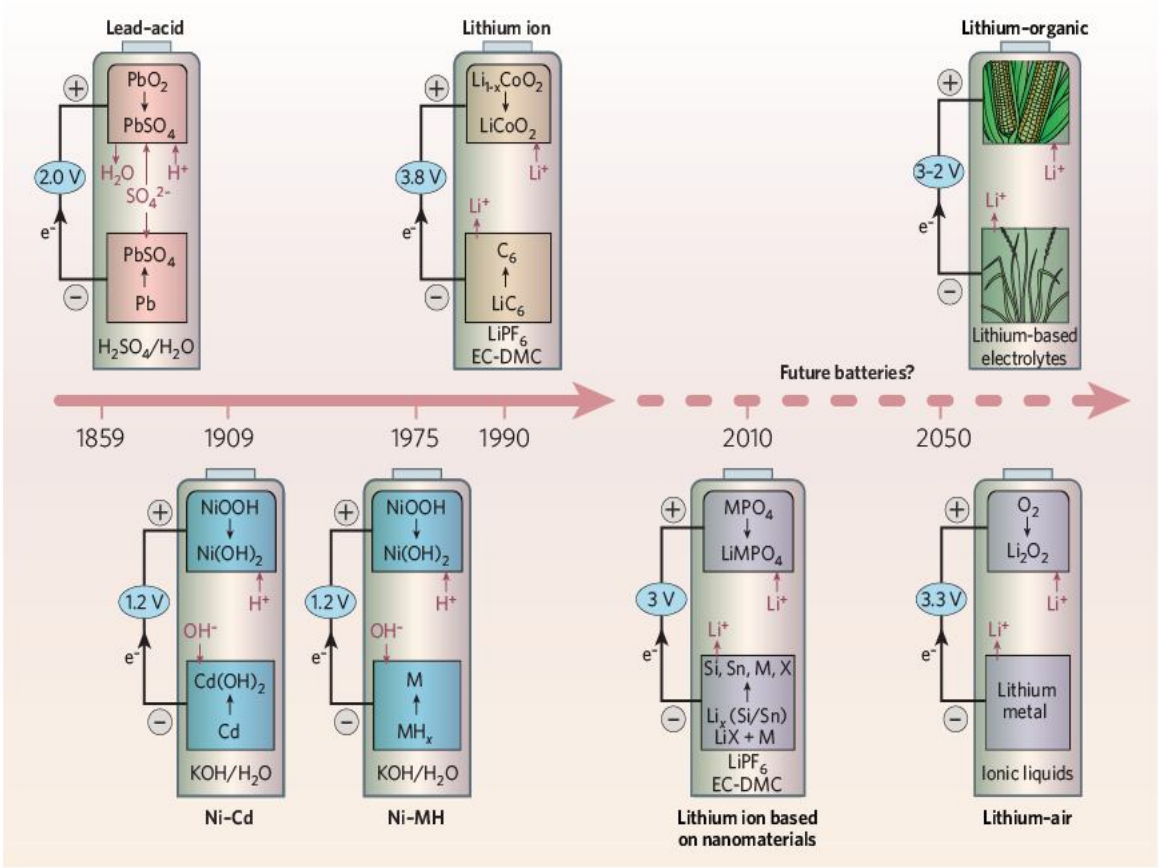



**Figure 2 | Battery chemistry over the years.** Present-day battery technologies are being outpaced by the ever-increasing power demands from new applications. As well as being inherently safe, batteries of the future will have to integrate the concept of environmental sustainability.

volume) of the reactants per exchanged electron as small as possible; and (3) by ensuring that the electrolyte is not consumed in the chemistry of the battery. This final condition was not true of the three principal battery technologies developed in the twentieth century, but holds for the more recent Ni–MH and lithium-ion batteries. One of the key elements of these two batteries is that the same ion ($H^+$ for Ni–MH and $Li^+$ for lithium-ion batteries) participates at both electrodes, being reversibly inserted and extracted from the electrode material, with the concomitant addition or removal of electrons. Ni–MH batteries are used to power hybrid vehicles and cheaper electronics, whereas lithium-ion batteries have conquered high-end electronics and are now being used in power tools. Lithium-ion batteries are also entering the hybrid electric-vehicle market and are a serious contender to power the electric cars of the future.

The lithium-ion battery, first commercialized by Sony in 1991, owes its name to the exchange of the $Li^+$ ion between the graphite ($Li_xC_6$) anode and a layered-oxide ($Li_{1-x}T^MO_2$) cathode[2], with $T^M$ being a transition metal (usually cobalt but sometimes nickel or manganese). The energy it stores (≈180 Wh $kg^{-1}$) at an average voltage of 3.8 V is only a factor of 5 higher than that stored by the much older lead–acid batteries. This may seem poor in the light of Moore's law in electronics (according to which memory capacity doubles every 18 months), but it still took a revolution in materials science to achieve it.

Billions of lithium-ion cells are produced for portable electronics, but this is not sustainable as cobalt must be obtained from natural resources (it makes up 20 parts per million of Earth's crust[3,4]). In addition, there are safety concerns, as the presence of both combustible material and an oxidizing agent carries a risk of runaway reactions resulting in fires or explosions. Improvements in the electrolyte composition could make the chemistry safer, but accidents are mainly a result of fierce cost-cutting and attempts to cram more active material in the same volume, causing internal short-circuits. As a result, improvements in monitoring and management are essential if lithium-ion batteries are to fulfil their potential in the automotive market.

Lithium-ion batteries would also need to reduce their carbon footprint, which is currently about 70 kg $CO_2$ per kWh (ref. 5). The carbon-related benefits of electric vehicles or 'plug-in hybrids' become apparent only after around 120 recharges with respect to electricity from coal, assuming a power-plant efficiency of 35% and that the batteries replace a petrol engine in which 20% of the heat from combustion is converted into useable energy. However, these break-even numbers need to be reduced.

Replacing each of the world's 800 million cars and lorries with electric vehicles or plug-in hybrids powered by 15-kWh lithium-ion batteries would use up to 30% of the world's known reserves of lithium. But lithium is also found in unlimited quantities in sea water[3,4], and concentrating it from brines is much greener (requiring just solar energy) than conventional mining. The demand for lithium could also be eased by recycling, which has already proved its value with lead–acid batteries. All these problems must be overcome if lithium batteries are to take their place as the batteries of the future (Fig. 2).

## The nanotechnology revolution

Most attempts to improve the design of lithium-ion batteries have tackled the problem at the macroscopic scale, but work is now focusing on the nanoscale. Nanomaterials were slow to enter the field of energy storage because the effective increase in the electrodes' surface area raised the risk of secondary reactions involving electrolyte decomposition. Only as recently as 2000 was it realized that such reactions could be controlled by coating the electrodes to protect the electrolyte from unwanted oxidation or reduction by the electrode materials. The arrival of nanomaterials gave lithium-ion batteries a new lease of life[6] and provided benefits in terms of capacity, power, cost and materials sustainability that are still far from being fully exploited.

Electrode kinetic issues can be circumvented by switching to nanomaterials combined with carbon 'nano-painting'[7], in which the grains are coated with a thin layer of carbon to bring the required conductivity to individual grains, whose small size shortens the diffusion path for ions and electrons. Moreover, by accommodating the strains associated with lithium insertion/removal reactions, as the volume can expand or contract several-fold, this has also made it possible to use materials with large volume changes on reaction with lithium, such as alloys. But there are pitfalls, the most important being the poor packing density of electrodes based on nanomaterials, which limits

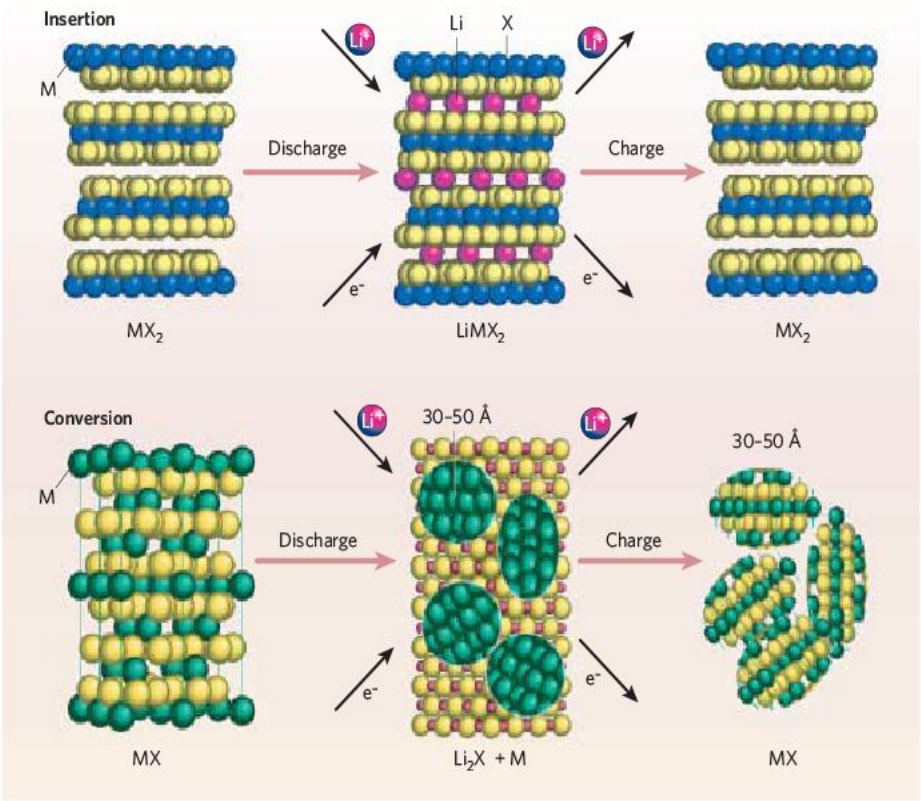



**Figure 3 | Reaction mechanisms.** Schematic representation showing the contrasting reaction mechanisms occurring during discharge for insertion (top) and conversion reactions (bottom). The insertion reaction demonstrates a maximum of 1 electron transfer per transition metal (here designated M), whereas the conversion reaction can transfer 2 to 6 electrons (derived from ref. 28).

the energy that can be stored per unit volume or mass because there is a larger proportion of 'inert' components such as current collectors or electrolyte.

Another advantage of nanomaterials is that they can change the reaction pathway, affording high capacities, rechargeability and generality to a range of battery systems[8]. One such reaction pathway is referred to as a 'conversion' from transition-metal oxides:

$$T^{M}_{x}O_{y} + 2ye^{-} + 2yLi^{+} \rightleftarrows x[T^{M}]^{0} + yLi_{2}O;$$

the final product consists of a homogeneous distribution of metal nanoparticles ($[T^{M}]^{0}$, where the superscript 0 indicates the metallic form) embedded in a $Li_2O$ matrix (Fig. 3). The drawback with this mechanism, however, is that the large voltage difference between charge and discharge results in poor energy efficiency. This problem is being addressed by studies of both the material chemistry and morphology and the electrode configuration. The hunt is also under way for materials that can undergo conversion reactions involving multiple electrons at high potential, for use as cathode materials. However, the full impact of nanomaterials on living cells has yet to be appraised, although the risk is minimal when they are produced *in situ*, as they are for the conversion reaction.

Solid electrolytes were quicker to benefit from the use of nanomaterials[9]. The addition of 'nano-fillers' (nano-grains dispersed in a polymer, such as $Al_2O_3$ or $TiO_2$) to simple polyether-based electrolytes increases the conductivity several-fold at 60–80 °C, but there is no advantage at room temperature. Organizing the polymer strands in such a way as to increase the order locally (using crystalline whorls, stretching or even chirality) can also provide benefits, by increasing conductivity at low temperatures, and further work is needed to assess the merits of using block co-polymers (AB or ABA)[10]. The phase separation inherent to these systems results in good mechanical properties, but also offers a way of increasing dissociation by partitioning anions and cations in the two sub-phases. Giving the two phases different wetting or adhesion properties can help by avoiding grain growth as the polymer's nano-domains will determine the partitioning of space.

True polymer batteries may still be some way off, but in the meantime we will see more attempts to use ionic liquids as either solvents for lithium salts or plasticizers for polyether-based electrolytes. Ionic liquids have exceedingly low vapour pressures, are non-flammable and have high conductivities, making them serious contenders for safer batteries. But it remains to be seen whether they can be produced cheaply enough, at the desired purity, with sufficient conductivity at low temperature.

## Beyond nanomaterials

The components of today's lithium-ion batteries, such as $LiCoO_2$ and $LiMn_2O_4$, are not produced from renewable energy resources but from ores, and extracting the raw materials and manufacturing the electrodes will require increasing amounts of energy as they become scarcer. Will the lithium-ion battery, which is so energetically expensive to fabricate, remain attractive and viable in the long term? In 50 years, if all cars become electric and rely on these scarce materials, might we face staggering price increases like those recently seen with fossil fuels? Not if we find a way of making lithium-ion batteries sustainable while maintaining or exceeding the performance of today's batteries. One option is to use renewable electrodes made from natural resources, just as fuel cells can use hydrogen or (m)ethanol made from biomass. But what would these electrodes be like?

## Inspired by nature

When scientists need new approaches, they often turn to the chemistry of life, with its virtually unlimited and incredible reaction mechanisms. The battery's insertion reaction may have no real equivalent in the living world, but the materials themselves could be fabricated in living cells. Phosphate species are manipulated to make DNA and ATP, so it is not so hard to envisage an enzyme-mediated synthesis of $LiFePO_4$, especially as the pH for the precipitation is close to the physiological value of 7. The same outlook applies to conversion reactions, as preliminary work[11] has demonstrated the synthesis of hydrated $Co_3O_4$ and $MnO_2$ with the help of a virus and a bacterium, respectively.

Perhaps the ultimate in conversion reactions also comes from living systems. The protein apoferritin, which encloses a small crystal of iron oxide ($Fe_2O_3.nH_2O$), can either grow or dissolve the particle according to the organism's current need for iron[12]. So could polymers with properties rather similar to proteins, adsorbed on the surface of a conversion electrode, control the growth or dissolution of the $T^{M}_{x}O_{y}$ and $Li_2O$ crystals, the reversible formation of which is key to increasing the energy efficiency?

Regarding the feasibility of using electrochemically active organic molecules as cathode materials, the use of polyaniline[13] and other redox polymers[14] has been much hyped over the years, but development has been disappointing. However, because lithium-exchanging materials do not involve the electrolyte in their redox processes, substituting the cathode for an organic material might boost the capacity. The feasibility of using active $Li_xC_6O_6$ organic molecules that can be prepared from natural sugars common in living systems (Fig. 4) is currently under investigation[15]. In the light of such findings, we can speculate on the use of hypericine (a polyquinone-based active ingredient of St John's wort) or the condensation polymers of malic acid as potential high-capacity cathode materials.

The close relationship between carbohydrates and their oxidized polyketone forms makes the former the logical starting point for the design of new electrode materials.

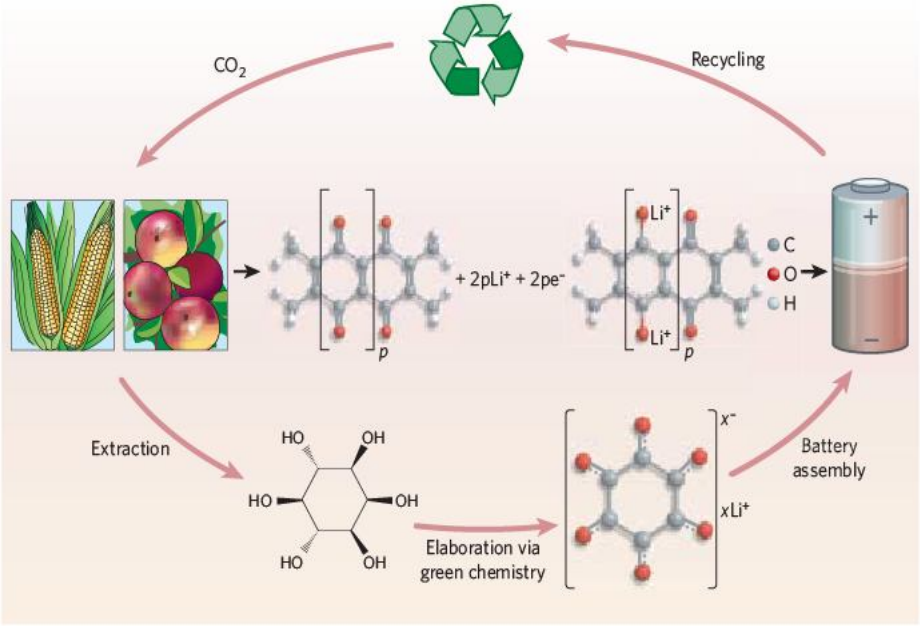



**Figure 4 | An organic future.** Proposed sustainable organic-based batteries based on electrode materials made from biomass. *Myo*-inositol extracted from corn can be used to prepare electrochemically active $Li_2C_6O_6$, whereas malic acid from apples can undergo polycondensation to a polyquinone that is electrochemically active to lithium (centre). (Derived from ref. 15.)

Polyketones can be obtained from natural sources and are unlimited because sugars can be made by living species or artificially in green chemistry[16]. Nor have sugars been overlooked for use in bio fuel-cells, a much improved version of which has recently been unveiled[17].

Although complex and unlikely to yield instant results, the search for electroactive organic molecules synthesized from biomass could pave the way for the next generation of lithium-based batteries. Organic materials have already made considerable inroads into the semiconducting industry, in light-emitting diodes, solar cells and transistors, and they are expected to penetrate the energy field in the coming decades. However, it would be foolish to ignore the fact that organic materials have several disadvantages in terms of their limited thermal stability, low specific gravity and appreciable solubility in electrolytes.

## Lithium–oxygen batteries

Air electrodes and metal–air battery technologies have already been used in primary systems such as fuel cells, but the use of lithium instead of zinc as the metal will increase the energy output eightfold. An oxygen electrode proceeding in tandem with lithium according to the reaction $2Li + O_2 \rightarrow Li_2O_2$ can deliver a capacity of 1,200 mAh $g^{-1}$. The first lithium–air cell was successfully assembled and discharged in 1996 (ref. 18), but attractive rechargeability was demonstrated only recently[19].

It could be argued that such a system unites within the same device the two most prominent failures of battery and fuel-cell technologies, namely the inability to master lithium and oxygen electrodes. These perceived issues have prevented the practical use of lithium–air batteries. However, one advantage of such a system is the formation of $Li_2O_2$ without cleaving the O–O bond, which has limited both kinetics and rechargeability in aqueous systems because there is a large activation energy and platinum catalysts are often required.

Improving energy storage and preventing $Li_2O_2$ from clogging the electrode require a better understanding of the reaction mechanism of the oxygen electrode. Engineering and chemical advances are also required to prevent the ingress of either $CO_2$ or $H_2O$, which could react with either $Li_2O_2$ or lithium metal. But there are reasons for optimism. The use of nanomaterials makes it possible to design porous, catalysed, three-dimensional electrodes[20] (Fig. 5) with improved kinetics and energy efficiency. The use of ionic liquids, which can be made hydrophobic, will put an end to problems caused by the entry of water. However, if ionic liquids are to be used as electrolytes, they must be combined with a highly hygroscopic Li salt, so preparing them remains a serious challenge.

Lithium has been the anode of choice for years, but much more work is still needed. When used with liquid electrolytes and gels, the metal is redeposited unevenly in the form of dendrites, leading to inherently unsafe cells with a short lifetime. It has been suggested that the cause lies in current inhomogeneities induced by the passivation layer present on the surface of lithium metal[21]. Using dry polymer electrolytes instead keeps the problem at bay for the first 600 cycles, but does not solve it. The classic strategy[22] to get uniform microcrystalline metal deposition in an aqueous solution from anionic complexes (for example, silver metal from $Ag(CN)_2^-$) has not been applied to the lithium electrode. In this strategy, the surges in local current result in a drift of the negative ions from the interface, leading to a depletion of the plating species and hindering the formation of metal dendrites. The same principle could be applied to lithium systems by using charged chelating complexes of the $LiX_2^-$ type, formed, for instance, using bidentate ligands of the 1,3-dione family (acetylacetone) and having $K^+$ as a counter-cation (see Fig. 6).

Another approach would be to use unipolar electrolytes, in which only the cations carry charge, but these have never been seriously studied in the context of plating lithium metal. This is surprising because polyelectrolytes with fixed negative charges attached to a macromolecule are the only way to avoid the depletion or over-concentration of salt arising from the

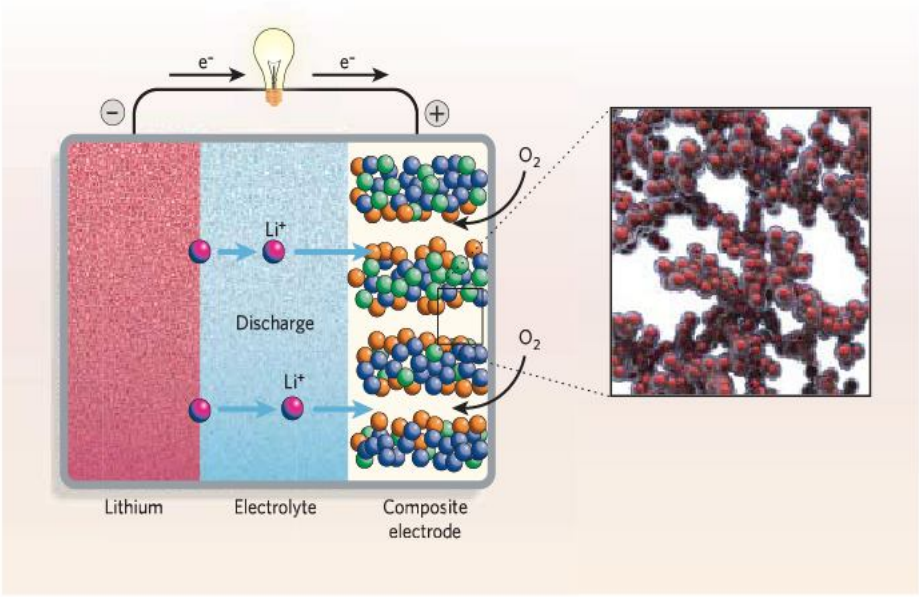



**Figure 5 | Lithium–air batteries.** Left, the mechanism used in lithium–air batteries (courtesy of A. Debard *et al.*, Univ. St Andrews). Right, three-dimensional nanoarchitectured electrodes made from depositing 10- to 20-nm-thick layers of $MnO_2$ onto a carbon foam using a low-temperature process (according to ref. 20) that could be used to enhance the kinetics of the lithium–air electrode (three-dimensional schematic courtesy of J. W. Long and D. R. Rolison, US Naval Research Lab.).

**Table 1 | Battery chemistries**

| Battery type | Features | Environmental impact |
|---|---|---|
| Ni–MH (established) | Low voltage, moderate energy density, high power density<br>Applications: portable, large-scale | Nickel not green (difficult extraction/ unsustainable), toxic. Not rare but limited<br>Recyclable |
| Lead–acid (established) | Poor energy density, moderate power rate, low cost<br>Applications: large-scale, start-up power, stationary | High-temperature cyclability limited<br>Lead is toxic but recycling is efficient to 95% |
| Lithium ion (established) | High energy density, power rate, cycle life, costly<br>Applications: portable, possibly large-scale | Depletable elements (cobalt) in most applications; replacements manganese and iron are green (abundant and sustainable)<br>Lithium chemistry relatively green (abundant but the chemistry needs to be improved)<br>Recycling feasible but at an extra energy cost |
| Zinc–air (established) | Medium energy density, high power density<br>Applications: large-scale | Mostly primary or mechanically rechargeable<br>Zinc smelting not green, especially if primary<br>Easily recyclable |
| Lithium–organic (future) | High capacity and energy density but limited power rate. Technology amenable to a low cost<br>Applications: medium- and large-scale, with the exception of power tools | Rechargeable<br>Excellent carbon footprint<br>Renewable electrodes<br>Easy recycling |
| Lithium–air (future) | High energy density but poor energy efficiency and rate capability<br>Technology amenable to a low cost<br>Applications: large-scale, preferably stationary | Rechargeability to be proven<br>Excellent carbon footprint<br>Renewable electrodes<br>Easy recycling |
| Magnesium–sulphur (future) | Predicted: high energy density, power density unknown, cycle life unknown | Magnesium and sulphur are green<br>Recyclable<br>Small carbon footprint |
| Al–$CF_x$ (future) | Predicted: moderate energy density, power density unknown | Aluminium and fluorine are green but industries are not<br>Recyclable |
| Proton battery (future) | Predicted: all organic, low voltage, moderate energy density, power density unknown | Green, biodegradable |

mobility of the anions. These two strategies (anionic lithium salts and unipolar conductivity) should be further explored to ensure that all avenues towards making the lithium-metal electrode viable have been exhausted.

## Alternatives to lithium

Although we have focused on lithium, there are several alternatives for use as electrodes (Table 1). The metals worth considering are magnesium (ref. 23) and aluminium (ref. 24) because of their light weight, but they deliver less voltage, undermining their use as anodes, and repetitive plating of these metals is difficult using most electrolytes. Similarly, only high-capacity cathode materials can be considered, which narrows it down to oxygen or sulphur for magnesium, or graphite fluoride for aluminium, to harness the metal's high affinity for fluorine. However, little is known about the kinetics of electrode reactions involving the motion of multivalent species, and addressing these challenges would need extensive collaboration between organometal researchers and electrochemists.

Proton-based battery technologies have been well studied, but do they still have anything to offer? Even with the best air electrode, to be competitive with lithium-ion batteries, a hydrogen system, with a voltage of 1.0–1.5 V, requires the anode to have an extremely low equivalent mass, far below that of conventional hydrogen-storing alloys. The only candidates are light elements, given that C–H bonds are too covalent and cannot (yet) be activated for reversible room-temperature systems. Another alternative for the negative electrode would be to exploit the reversibility of the N–H bond in semiconjugated polymers (see Fig. 6). These low-potential ($V$ versus $H_2/H^+$) materials could be used as high-capacity electrodes, although their low electronic conductivity could prove problematic.

## Miniature powerhouses

As well as large-scale applications, such as electric vehicles, batteries must also be developed to satisfy recent advances in microelectronics. These require miniature power sources, such as solid-state, lithium-based, thin-film batteries. Much of the work has focused on flat, two-dimensional configurations, but these are limited in terms of energy output, and the need for greater performance has recently led microbattery researchers to explore the third dimension[25]. This might seem relatively easy, given the spectacular three-dimensional circuitry that the silicon microelectronic industry now has to offer. However, microlithography processes have proved both awkward and costly to transfer to batteries.

A combined chemical–electrochemical approach has much to offer as a way of manipulating materials at the atomic scale, and could be used to develop 'skyscraper' batteries (Fig. 7). Similarly, adding a third dimension opens the way to a larger variety of configurations (such as the assembly of positive–negative electrodes and electrolyte) while maintaining a short diffusion length for electrodes and ions, which is essential if a battery is to have the required power.

## Conclusions

It is not yet clear whether the next generation of batteries could be successfully integrated into an energy market that is currently linked to global warming. Fame and fortune certainly await anyone who can come up with a viable alternative to fossil fuels. Furthermore, it is difficult to see how the performance gap between the internal-combustion engine and lithium-ion batteries will be filled using only new battery technology; other approaches, such as fuel cells, will be needed, but here a complete overhaul of present systems will probably be required.

In our journey into the future we have reinvestigated existing systems and suggested new trends and ideas that require much work to become a reality. Designing green and sustainable battery systems is essential, so criteria such as life cycle, abundance of raw materials and electrode recycling are becoming crucial. For these reasons, much is expected of the lithium–air system, which offers a great improvement in energy density, and lithium-

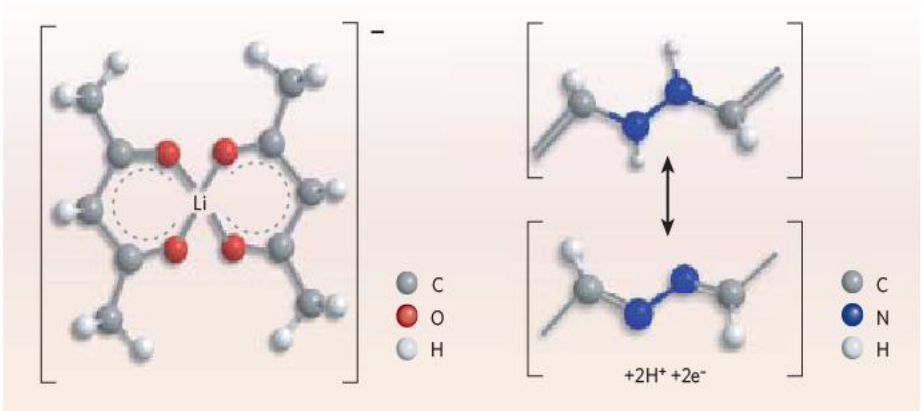



**Figure 6 | Wild cards.** Lithium-bearing anionic complexes that could be explored for efficient lithium plating (left) and a contender for a high-capacity proton-exchanging polymer (right).

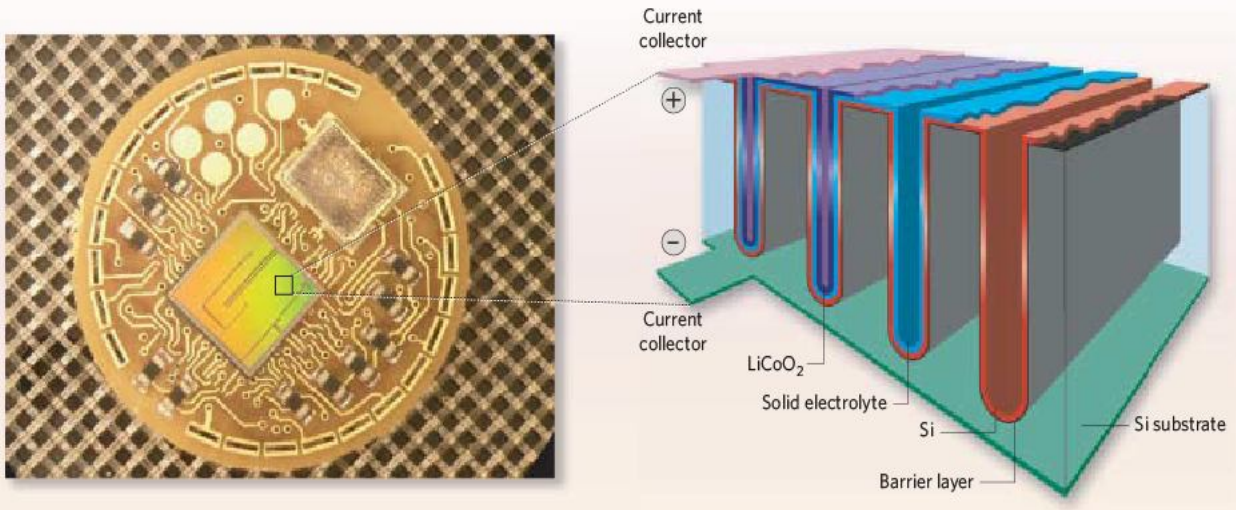



**Figure 7 | Entering the third dimension.** Schematic representation of a three-dimensional, integrated, solid-state lithium-ion battery. The surface area of the battery has increased 25-fold compared with a two-dimensional thin-film battery with the same footprint surface area, and will therefore be able to provide enough energy to power smart autonomous network devices related to sensing applications. (Courtesy of P. H. L. Notten, R. A. H. Niessen and L. Baggetto, Philips Research Laboratories, and Technical University of Eindhoven, the Netherlands.)

based systems that use electroactive organic molecules, which could be obtained from biomass using green chemistry. Yet it seems incongruous to insist that batteries are sustainable while the car or appliance they drive is not.

The next generation of lithium-ion batteries fully based on nanomaterials will soon be here, followed by lithium–air batteries and others using organic materials. And there is plenty to inspire us in the living world, as long as we can capture the function of each molecule in a cumulative sequential process, which is not an easy task. Both biofuel cells and high-voltage liquid-electrolyte microbatteries inspired by electric eels have already been demonstrated. We all live on organic-based energy, so why shouldn't our appliances and vehicles use it too?

One thing is clear, however. Solving the remaining challenges will require researchers from a range of disciplines, and their success will depend on the efficiency of their cross-fertilization. ■

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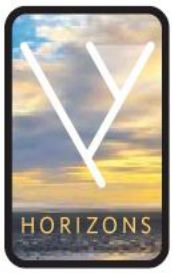
# Evolution of anatomy and gene control

Georgy Koentges

**Evo-devo meets systems biology.**




We've probably all heard the story of the six wise men left in a darkened room with an elephant and asked to say what it was. Each felt a different piece of the elephant, compared it to something else and interpreted the whole as a simple extension of the part he described. There was no common observation ground, so nobody recognized that each description just captured some significant aspects of the same object[1].

The delightful Persian miniature of a composite elephant at the Aga Khan Trust for Culture in Geneva (Fig. 1) conveys such a concept. We understand that living things, such as the elephant, are complex and, fortunately, there is a bit more light around these days. Palaeontology, developmental biology, gene regulation and systems biology gather around the beast and use their tools to illuminate its parts. But we have to figure out how the parts relate to each other, and how we can communicate about them appropriately.

Since Darwin we know that we must explain the elephant not only in mechanistic terms (of mutation, selection and adaptation on the population level) but also in historical terms, as 'descent with modification', evolution in phylogeny. Molecular changes hundreds of millions of years ago constrain the possibility of change here and now. Not everything is possible, and evolutionary history is as much a story of constraint as functionality. Leonardo's 'flying machines' didn't just fail because bodies of a human size and weight fall under physical scaling laws limiting how big muscles could become. The evolutionary history that led to our present body size also stops us acquiring wings, either now or any time soon.

We know that we are constrained by genetic baggage, but the molecular causes have remained elusive. They vacillate between being neutral and adaptive at different times in our phylogenetic history[2], so purely functional studies offer little prospect of finding them. Comparative approaches of molecular function can reveal them, as an elegant study on the darwinian evolution of ligand–receptor interactions has recently illustrated[3]. Because phylogeny is a concatenation of developmental processes in populations, all heritable morphological changes derive from developmental changes in molecular control hierarchies and networks[4]. The daunting task of the field known as evo-devo is to map structural diversity onto the underlying gene-regulatory diversity and dynamics.

**Figure 1 | Illuminating the elephant of complexity.** This composite elephant is from a Persian painting from around 1600. (Courtesy of the Aga Khan Trust for Culture, Geneva.)

Developmental regulatory changes have affected patterning, differentiation and growth. Patterning describes the highly regulated, three-dimensional self-organization of groups of embryonic cells towards structures we can see. Differentiation is the allocation of cells to particular fates, such as muscles and bone. Differential growth implies that certain (molecularly defined) groups of cells grow more rapidly than other groups within an organism. Developmental genetics has shown that these three activities are often linked in complex ways but are separately controlled molecularly in space and time.

The embryonic locations of such linkages are genetically defined cell lineages, where the molecular actors — the genetic control networks — must have changed their plots to create phenotypic diversity. Historians of life are interested in the specific succession of character changes as they happened over evolutionary time. By joining forces with mechanistic disciplines, they can learn how to read visible characters as epiphenomena of the most information-rich units within biological structures, and apply this knowledge to understand fossil anatomy. Such information-carrying units are not accessible through intuition: only genetic experiments allow us to see the elephant from the inside. Comparative genetic analysis tells us that the elephant consists of many parts that are also used by other organisms for both similar and different purposes, and that the differences between the parts' connections contain valuable information.

These 'atoms' of biological information are hard to measure. Each scale of organization requires different descriptors, and it is difficult to conceptualize how single-molecule dynamics on two strings of DNA (in a diploid organism) can cause major structural changes over historical timescales. Systems biology is starting to make it easier for those speaking the languages of DNA and mathematics to interact, under the auspices of massively parallel measurement platforms, comparative genomics, graphical models[5] and dynamic systems theory[6].

Here I will outline how introducing historical information into the mechanistic fields of developmental biology, gene regulation and systems biology can stimulate useful new dialogues and exchange of expertise. These disciplines can teach — and constrain — each other about where and how to look at the unique features of living information-carriers and build common observation platforms. By applying genomic width, mechanistic precision and historical depth, such approaches will help us describe our ideas in less-intuitive but mathematically sound ways, in a language that machines can process. This may enable us to slowly make out the immensely rich historical contours of the elephant of complexity as it emerges from the darkness of time.

## Charting metazoan history

Palaeontology is equipped with powerful statistical tools to reconstruct phylogenies, and a sophisticated armoury of non-invasive structural investigation techniques to trace the succession of structural changes as they happened during history. Phylogenetics allows us to reconstruct trees of ancestral relationships using the rules of parsimony (favouring the fewest evolutionary changes) and synapomorphy (by using unique character states shared by two groups assumed to be inherited from a common ancestor)[7]. Other characters, which are not used for phylogeny reconstruction, can then be charted onto these trees, revealing the direction of changes across vast historical timescales (Fig. 2).

Phylogenies put extant species in the appropriate historical context and reveal which characters studied in live organisms are really 'ancestral' or have significance for a larger taxonomic group. Thus insights from molecular and developmental biology rely on a foundation established by morphological and palaeontological studies.

Fossil studies are in turn starting to benefit from knowledge of the molecular players behind the characters. Palaeontology reveals combinations of fossilized characters that are no longer seen in extant organisms, but that shed light on the evolution of development and complexity. For example, the evolutionary emergence of the vertebrate skeleton was recently redefined in a profound and non-intuitive way[8] (A in Fig. 2). An anatomist comparing living sharks with bony fishes would conclude that a dermal bone cover is a feature unique to bony fishes. A palaeontologist, in contrast, discovers that the common ancestors of sharks and bony fishes had extensive, bony body armour (B in Fig. 2). In this light, the absence of such armour in sharks must be reinterpreted as a secondary loss (C in Fig. 2), with its presence in bony fish being the retention of an ancestral state.

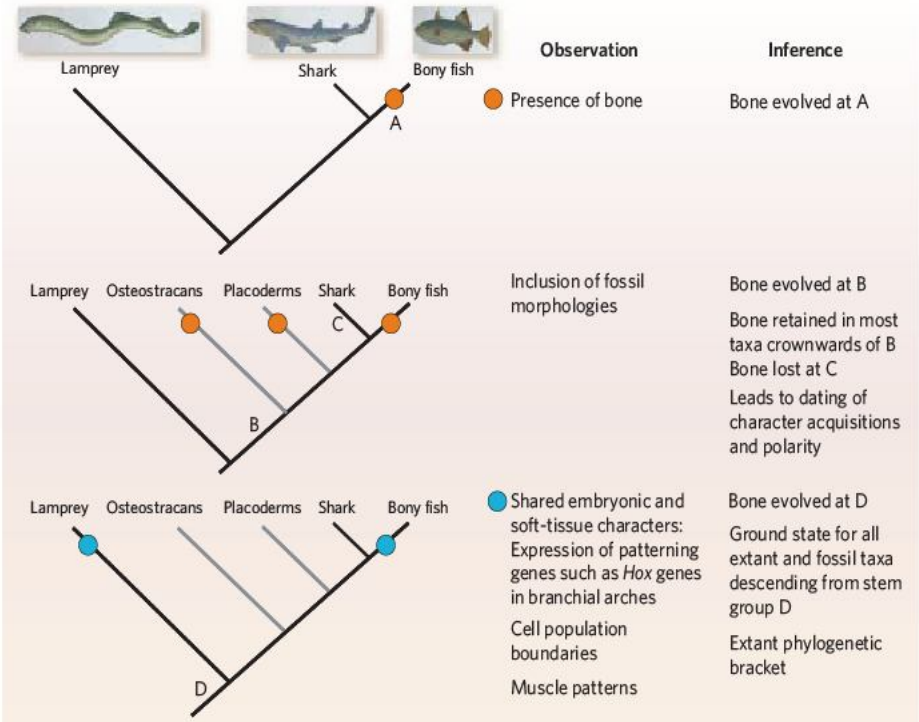



**Figure 2 | Character evolution and gene expression in fossils.** Soft-tissue and molecular characteristics of fossils can be inferred from their respective phylogenetic position by using the 'extant phylogenetic bracket'[9].

This purely palaeontological discovery has dramatic consequences for any line of enquiry that a developmental biologist who studies the molecular evolution of skeletogenesis might wish to initiate. A simple genetic comparison of skeletogenesis between extant sharks and bony fishes would not be meaningful, at least in terms of understanding the sequence of historical events that led towards having (or not having) a skeleton, as secondary bone loss might not involve the same molecules responsible for acquiring skeletons in the first place. Historical information is thus indispensable for refocusing the efforts of researchers who could otherwise go astray by trying to find molecular explanations for evolutionary processes that have not taken place but were incorrectly inferred from incomplete extant character distributions.

Studies of character evolution are currently flourishing, thanks to new tools and discoveries. Beyond this, historians of life have a more fundamental agenda. They like to assign structural, cellular and molecular 'ground states' — combinations of old and new characters — to particular nodes of the phylogenetic tree. This is initially a sorting and classification exercise within robust phylogenies, but the idea behind it is more profound: mapping observable, phenotypic range onto an underlying map of inferred molecular diversity. A useful inference tool for this purpose is the 'extant phylogenetic bracket'[9] (Fig. 2, bottom). If a group of extinct forms can be 'bracketed' phylogenetically by two extant forms, the similarities between these extant forms are likely to be a common heritage of the entire monophyletic group (including the bracketed fossils) and can be used to define the direction of molecular changes among these fossils. Although fossils cannot be genotyped, their genotypic composition can be inferred from their precise position in phylogenetic trees with respect to extant forms that can be genotyped.

This bracket criterion is applicable to any soft parts or molecular characters and can, for example, be used to infer cell-lineage fate maps or ancestral gene-regulatory networks in fossil forms. This powerful method has rarely been used because no databases or data formats yet exist that allow phylogenetic information to be collated in such a way as to allow all comparative genomic data to be assigned to specific phylogenetic nodes. Ideally, we could go into such a database, pick a particular phylogenetic tree position and obtain all the information inferred from phylogenetic bracket comparisons of entire genomes. This could then be correlated with morphologies, if such a database contained images of all the precious objects locked away in collection drawers. This would also institutionalize efforts to reconstruct ancient genomes and provide an appropriate comparative reference point for experimental analysis of the gene-regulatory circuitry.

Surprisingly, fossils can help in charting the evolution of gene controls affecting tissues.

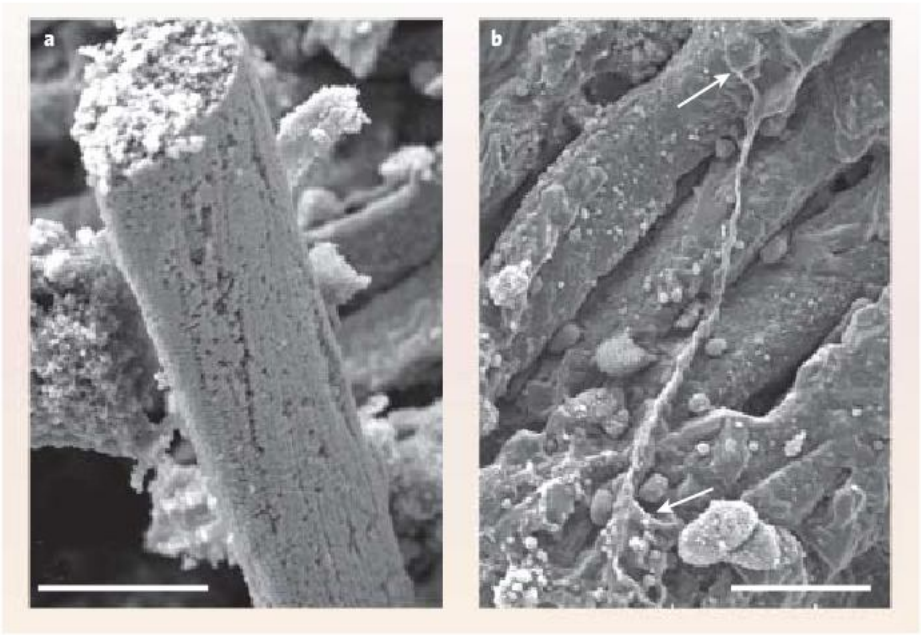



**Figure 3 | Tissue preservation in fossils.** **a**, Exquisitely preserved histology of muscle fibres with Z-banding and **b**, motor-axon endplates (arrows) in a 380-million-year-old placoderm. Scale bar: 20 μm. (From ref. 10, courtesy of the Royal Society.)

Some fossils recently discovered exhibit pristine tissue preservation down to the submicrometre level, offering rich histological and cellular information about organisms that died hundreds of millions of years ago. For example, on some fossil placoderms — members of a wholly extinct group of fishes akin to the common ancestors of sharks and bony fishes — even the neuronal processes of motor-axon endplates on muscle-fibre Z-bands are still visible[10] (Fig. 3, arrows).

Palaeontologists have recently used synchrotron X-ray phase-contrast tomographic microscopy and image analysis to study three-dimensional anatomy and histology non-invasively[11]. The richness of structural data that can thus be extracted from fossils and assigned to phylogenetic nodes is remarkable, and can, in some cases, match the subcellular scale of the analysis of mutant extant organisms. Fossils have the added benefit of a temporal perspective, as they potentially allow the precise dating of the underlying molecular pathways responsible for histogenetic processes and their evolution.

If palaeontologists are to 'read' these historical phenomena appropriately, they will have to interact with developmental cell biologists well versed in the molecular pathways involved. Pinning the components of molecular pathways onto phylogenies is no trivial exercise, owing to the pleiotropic nature of gene and pathway action. The modularity of genetic traits or pathways encourages us to associate histological changes with regulatory phenomena accessible through comparative genomics.

New databases that integrate images along with phylogenetic and molecular information would enable new synergies across disciplines. One would be able to investigate evolutionary novelties and convergent trends in a multidimensional search space. Structural or molecular bottlenecks of robustness and system fragility[12] that govern cellular state transitions in more than half a billion years of evolving metazoan ontogenies could become obvious, if those in a dimly lit room chose to join forces and build better lamps to illuminate their precious elephant.

## Single cells in lineages

Cell lineage is the cause of functionally specialized cells in all multicellular organisms. It implies that molecular decisions made in a (mother) cell at a particular position in the developmental lineage are inherited by daughter cells, despite the diluting effects of cell division on gene-regulatory components.

Mother cells of particular lineages make molecular choices that affect three key phenomena: patterning, differentiation and growth (Fig. 4). Patterning information is laid down as 'address codes' of positional identity in the embryo, where each cell's (and its daughters') responses are set in a collective manner and affect a wide variety of behaviours leading to three-dimensional shapes and connectivities (green in Fig. 4). Cell differentiation commits cells to a particular fate, irrespective of their positional information (yellow and purple in Fig. 4). Finally, the growth of particular lineages affects the elaboration of particular descending structures in size and shape through cell divisions.

Patterning, differentiation and growth become apparent at different times, are laid down in the embryo along different spatio-temporal axes, and are implemented by different genetic regulators, often through complex combinatorial coding schemes. Sometimes these coding schemes have direct anatomical read-outs that palaeontologists can trace through history. At other times these key molecular units are transitory scaffolds that are removed after morphogenesis, in which case their presence and significance can be revealed only by genetic lineage analysis.

In the past three decades, developmental genetics has repeatedly shown how our intuitive preconceptions about lineages and their distributions in adults can mislead us, whether we look at segments in insects[13] or the segmental[14] and non-segmental[15] structures in the vertebrate brain, head[16] and neck[17]. We do not know in advance which anatomical characters will reveal the most about the underlying cell lineages. Mapping them genetically and historically, across evolution, represents a formidable intellectual and technical challenge.

Such genetic-fate mapping can extract informative morphological details that form the basis for a comparative analysis. When experimental embryology using cell transplantations reached its technical limits, genetic-fate mapping in transgenic organisms was made possible by using recombinase enzymes[18] that can perform permanent genomic modifications in particular embryonic (and adult) lineages, rendered visible by genetic reporter activities[17] (Fig. 4). This incredibly powerful tool allows us to link the earliest genetic lineage decisions directly with final morphology.

Curiously, this technique has only rarely been used to address evolutionary questions. Evolutionary change in embryonic development can occur in either of two ways: if there are changes to the signals that a spatially and temporally invariant lineage in the embryo receives; or if the spatial extent and identity of a given lineage itself changes. Genetic lineage labelling provides an excellent way of discriminating between these possibilities. To perform such comparisons, homologous genes and their regulatory regions (which are indicative of particular homologous lineages) need to be used experimentally in a variety of phylogenetically relevant taxa. These must be of sufficient age and phylogenetic distribution that statements of homology can hold for all organisms concerned (blue in Fig. 4).

The current resolution of attempts to label primary embryonic lineages barely goes beyond the tissue types recognized by classical embryologists. However, as every single descending cell of a given lineage is labelled, we can make strong inferences if these cells end up at interesting places and display interesting behaviours, with 'class' properties becoming measurable. Such work can reveal that patterning — for example in connectivity between structural elements — remains constant, whereas the patterns of differentiation and growth change[16,17,19]. However, fate-mapping more subtle molecular subdivisions in embryos, which can be studied in a sufficiently diverse number of taxa, is currently beyond us.

Efficient transgenesis has been the most arduous obstacle to date, at least in vertebrates. The latest advances in transposon[20], meganuclease[21] and lentiviral[22] transgenesis promise to broaden the range of species that are amenable

to genetic labelling with single-cell precision. It is in single cells belonging to genetically defined homologous lineages that we need to study homologous gene networks and regulatory components and their evolution (Fig. 4).

## Evolution of gene regulation

The central players of evolutionary change are likely to be elements of the gene-regulatory machinery, transcription factors and their cognate genomic binding regions, which are clustered in '*cis*-regulatory' modules (CRMs) and promoters[23]. Little evidence has so far emerged for a role of chromatin or small RNAs in evolutionary changes of morphology, but this may be an artefact of observational bias. Ultimately, major morphological changes can be viewed as epiphenomena of dynamic changes in RNA–Pol II holoenzyme complexes (HECs) acting on regulatory gene nodes in key morphogenetic circuits (Fig. 5). This idea has been refined over the years in cogent discussions of 'evolvability'[24], but there are few specific examples in metazoans where we can assign major structural changes to specific gene-regulatory causes.

Evolutionary 'tinkering' has occurred on many regulatory levels by the acquisition or loss of network components or changed functionalities. During the evolution of the wing gene regulatory network in various ant species, for example, the loss of key network components has occurred independently several times[25]. Existing networks can be co-opted for new patterning purposes at different places in the embryo. During the evolution of fins in early vertebrates, a pre-existing programme of nested *Hox* gene expression in the unpaired fins of agnathans was co-opted to a new embryonic location (the recently evolved paired fins) in the early jawed-vertebrate stem lineage[26]. Similarly, the evolution of promoters was implicated in the acquisition of new *Hox* expression domains in the mesoderm between agnathans and jawed vertebrates[27].

Regulatory networks can also be redeployed at similar places later in development, such as the insect appendicular patterning system that later becomes responsible for wingspot patterning in butterflies[28,29]. Occasionally, evolution can occur within the regulatory proteins themselves: the arthropod *Ubx* homeobox gene, for example, acquired a protein domain responsible for the loss of abdominal legs in insects, in contrast to its homologue in the crustacean-like ancestor[30]. Most of these phenomena were due to genes being turned on or off, or being present or absent. Improved transgenesis tools and comparative genomics will soon allow us to test regulatory network components functionally in a more quantitative manner.

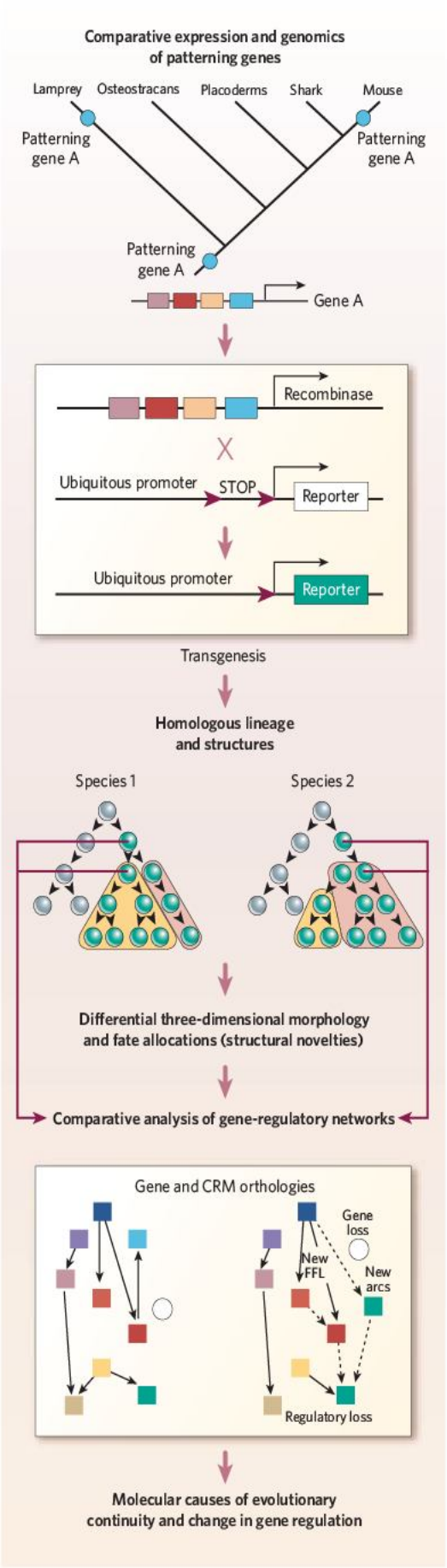



**Figure 4 | Evolution, development and systems biology.** These map the changes in information flow through biological systems across scales of organization. Phylogenetically conserved regulatory regions are used to map pertinent embryonic lineages. Expression profiling of those lineages allows the investigation and inference of the regulatory networks underlying their behaviour.

The main technological problems are currently being solved, clearing the way for truly genomic systems analysis of morphological evolution that can go beyond examples of ephemeral beauty. Methods now exist for the transcriptome-wide profiling of single cells in particular lineages at specific places in embryos, using a combination of laser-capture microdissection, single-cell cDNA synthesis and microarray analysis[31]. In combination with genetic lineage labelling, these methods allow us to determine the minimal regulatory toolkit used by lineages at key morphogenetic stages.

Although comparative cDNA measurements have provided fundamental insights into the evolution of beaks in Darwin's finches[32], for example, current array-based platforms are rather inflexible and costly. They are likely to be replaced by second-generation massively parallel sequencing technology[33], which opens up transcriptome analysis to new species: entire cDNA libraries could be sequenced expeditiously and digital information about differential RNA abundance in homologous single cells obtained, irrespective of their species origin.

These experimental platforms turn a previously insurmountable task into a computational mapping exercise that can take advantage of our ever-growing pool of sequence-homology information. Probabilistic methods[5,34] can then use this information to infer network similarities (and differences) in homologous lineages — something already in our reach. To avoid bias for genes and pathways well-known to biologists, we need the independence of statistical network analysis. Automated network inference[35] needs to be constrained by additional information, such as the presence of statistically significant transcription-factor binding sites on CRMs[36].

The speed of CRM discovery by comparative genomics has been accelerating ever since a larger number of phylogenetically interesting taxa were sequenced in their entirety[37]. CRMs are being detected with ever-increasing coverage, and dramatic improvements in the detection of true CRMs with a conservation score below 75% are still to be made.

CRMs determine the place and timing of gene action[38]. Current estimates for CRM numbers in vertebrates are in the few thousands, but this is probably a significant underestimate (G. K. and S. Ott, unpublished data). In CRMs, genes measure transcriptional inputs in ways we do not yet understand. CRMs are likely to act as logic functions[39], coincidence detectors, filters, gradient sensors and resistors, all of which ultimately influence the kinetics of activators, repressors, SRB/mediator complexes and Pol II-HECs, grouped in 'factories'[40].

Because HECs have not changed much structurally, the major structural source of

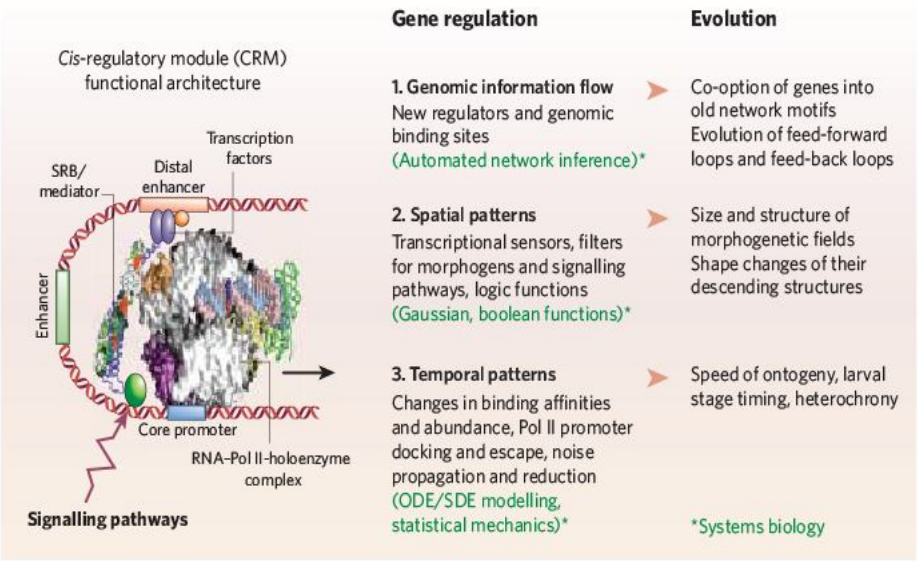



**Figure 5 | Gene regulation as a driver of morphological change.** The mechanistic core of gene control in evolving embryos and structures is shown.

evolutionary novelty is likely to be found in the composition and action of the CRMs themselves. To begin with, these need to be classified bioinformatically. We also need to develop more[39] statistical methods that can take into account the phylogenetic conservation of the order of binding sites within CRMs, to highlight the core architecture of 'enhanceosome complexes'. For now, it is not clear whether 'enhanceosomes'[41] (where the order of binding sites matters due to synergistic, orientation-specific transcription-factor binding) or 'billboard enhancers'[42] (where the order of transcription-factor binding sites matters little) are the more frequent; this would shed light on our ideas of evolutionary novelty, as temporal changes are known to affect spatial expression patterns. The most famous example is the structural co-linearity of *Hox* genes and their temporal and axial deployment in vertebrates[43,44].

Any functional classification needs to capture spatial and temporal aspects of gene activation in relation to these CRMs. This is tedious and technically difficult to do as it requires the combinatorial cloning of CRMs and slow transgenic assays in cells or organisms. Fast transgenesis protocols are now available for invertebrates such as arthropods, sea-urchins[45] and sea-squirts[46]. Recent studies on vertebrates suggest that only a fraction of 'ultra-conserved' CRMs are active and absolutely required for the animal to survive[47]. As CRMs were assayed at only a few embryonic time points, the absence of regulatory information cannot be construed as a lack of function. It is not clear when, and in which cell types, organ-system specific CRMs are expected to be active, so inferences from such studies should be treated with great caution. We have no idea what compensatory mechanisms will kick in to obviate the regulatory bottlenecks when CRMs are experimentally removed. Even the genomic removal of expected key CRMs belonging to *MyoD*, a master regulator of myogenesis, had only a moderate effect on the timing of gene expression[48], suggesting that the CRMs might be interacting in complex ways. Some CRMs might modulate the activities of others, so their effects might not be apparent unless their activity is assayed in a combinatorial fashion over time. We need to develop combinatorial strategies involving more subtle loss- and gain-of-function testing of CRMs if we are to reveal such regulatory logic comprehensively and comparatively.

## Genomic systems biology

Transcriptional regulatory networks can be subdivided according to the way information flows[49]. By analysing recurring network motifs of gene regulation among living species in a phylogenetic framework (functional testing has not been done for metazoans), we can compare their 'computational function' and trace their evolution. How did certain transcriptional inputs at one point of the network lead to certain outputs (numbers of RNA molecules) at another point, and how did this change? In insects, certain upstream 'selector genes' not only activate batteries of intermediate targets but also act directly on most downstream genes that encode structural components such as photoreceptors or pigmentation enzymes, much as the chief executive of a company talks to the secretary[50].

This constitutes a 'feed-forward loop'. In many cases, such as the *Ubx*- or *Pax6*-dependent[51,52] networks that underlie wing and eye development and evolution, the communication between chief executive and secretary was the starting point, with the feed-forward loop developing later. When did this happen, how often and why? Studies of prokaryotes have suggested that feed-forward loops have specific computational functions[49], but these need to be explored functionally in metazoans and in a comparative manner.

Until this happens, talk about 'co-option' or 'homology of regulatory cassettes' is premature. Expectations about 'master regulators' (of processes such as gastrulation, neural-crest formation, skeletogenesis or eye formation) may have to be reassessed; the chief executive might leave and a new one join while the company remains unchanged, or the other way round. In vertebrates the expression domains of most patterning 'master regulators' have not changed significantly since agnathan times, despite obvious signs of morphological evolution.

This does not mean that targets or motif dynamics have remained unchanged. We do not have the data to see static (let alone dynamic) differences in network motifs in a comparative manner. Motif deployment and speed will play key roles as developmental systems biologists try to observe these gene-regulatory circuits in action (Fig. 4).

Although information about spatial regulation can be obtained from whole embryos, temporal regulation through CRMs is better studied in judiciously chosen single-cell assays. Advances in massively parallel imaging and tracking assays could yield sufficient temporal resolution for modellers to survey a host of functional input–output functions, a question at the cutting edge of genomic systems biology. Such assays will allow us to test CRMs of different species and check whether temporal regulation might have evolved and, if so, whether it can be ascribed to CRMs directly, as opposed to transcription factors or the speed of the activator, repressor or RNA–Pol II HECs occupying them. Such speed differences in homologous network motifs will provide valuable insights into the causes of evolutionary heterochrony if they are focused on those genes deemed relevant in a dialogue between development and palaeontology.

When evolutionary biologists have inferred the composition of ancestral CRM sequences and transcription factors, we can use mathematical tools of systems analysis to formulate the dynamics of their action, capturing both deterministic and stochastic effects[53]. The dynamics at each gene network node are based on a few molecular complexes sitting on, and sliding along, two molecules of DNA (in a diploid organism), probably in non-equilibrium states. They are therefore subject to significant stochastic, mesoscale effects, and it is rather surprising to see any deterministic behaviour at all in patterning over developmental and evolutionary timescales.

Determinism causes robust morphological outcomes and enables us to establish anatomical homologies in the first place, so surely well-confined stochasticity needs to be the source of change. If the highly conserved regulatory apparatus that acts on a fundamentally stochastic background is expected to be central for spatio-temporal regulation, it is surprising to see considerable differences in the size of embryos (and morphogenetic fields). Noise tolerance[53] for key regulators is likely to differ, depending on whether it is expressed by a thousand cells or a single cell, or is dependent on the organism's ploidy status. Bigger morphogenetic

fields might be expected to afford less accuracy, but do they? When were key noise constraints laid down in history, and how big were the morphogenetic fields in the embryos of ancestors of major phylogenetic groups?

Until we can measure the noise of homologous network nodes in a variety of species, these systems questions will remain unanswered. Interspecific swaps of network components, assayed in live single cells or organisms, will help us find the basis of evolutionary novelty. Differential ontogenetic speed is a likely source of experimental difficulty, as indicated by recent transgenic studies combining *Hox*-gene CRMs and promoters from different species[54].

Recent advances in massively parallel synthetic DNA chemistry[55], currently used to construct microorganismal genomes from scratch, will one day allow us to synthesize thousands of CRMs belonging to ancestral genomes directly. This is not *Jurassic Park* (yet), although advances in genome engineering may one day let us test a plethora of such ancestral CRMs simultaneously and functionally in living cells and organisms. Such assays will be necessary to assign evolutionary fitness functions to specific CRMs and transcription-factor binding sites within them[56], and to help us formulate intragenomic constraints, such as competition of different CRMs for RNA–Pol II factories, which are currently below the radar of genomic systems biology and its models.

## Stochasticity in evolution

By establishing common measurement and analysis platforms, palaeontology, developmental biology, gene regulation and systems biology can each make unique contributions towards explaining the history of life (Fig. 5). Palaeontologists can reconstruct phylogenetic trees, determine structural and molecular 'ground states' at key tree nodes, and discover evolutionary directionalities. This allows them to work out what needs to be explained mechanistically. Developmental biologists, armed with their genetic toolkits, can identify the smallest units of information within development as lineages whose alterations lead to the observed anatomical changes, and refocus the historian's attention onto the most revealing anatomical details.

Making homologous lineages the reference point for studies of gene regulation will allow us to identify shared regulatory network nodes and motifs[49] and to measure their dynamics *in vivo* and *in vitro* (Fig. 4). With the help of synthetic DNA chemistry[55], inferred historical genomic information can be tested functionally for its effects on gene regulation in live cells and organisms. Systems-biology studies of network sensitivity and noise propagation[57] can then help to formulate the dynamics of deterministic and stochastic components[53]. The latter will identify 'hotspots' of systems' flexibility that are relevant to morphological evolution. Direct experimental manipulation of such hotspots might then enable us to 'replay' key evolutionary processes within the lifetime of single experimental organisms.

There might be some initial disappointment that nature neither constructed its regulatory circuits with an engineer's intelligence nor used Occam's razor, whereas we must use both to describe it. Historians can help us decipher when and how new information was introduced, and into which old (and imperfect) information networks. As information networks are corridors of constraints, they depend on the states of their predecessors, subject to modification by stochastic forces. By placing the robustness and fragility[12] of regulatory systems in a historical context, palaeontologists can identify phases of ontogenetic 'experimentation' on a larger phylogenetic timescale, at the base of the major metazoan radiations, and identify the key players and the (changing) rules in deep time.

The links between evolution and systems biology are tenuous at the moment because of limitations in what we can measure. An elegant recent study about tooth patterning that links the mathematical modelling of signalling pathways with the comparative analysis of evolutionary diversity points in such a new direction[58]. Only a few metazoan systems are known in which noise is harnessed to establish phenotypic diversity (for example, in *Drosophila* photoreceptor choice[59]) or to capacitate evolutionary change (such as *Hsp90*)[2]. This shall not discourage us. Powerful tools will be available to assess the effect of a few regulatory molecules in non-equilibrium states[60] on whole-organism structure and evolution.

We can now see more than Darwin could ever have imagined. Comparing species genetically helps us see similarities and differences on each organizational level. The analytical tools are almost in place to integrate data sets into an edifice of human knowledge that transcends our inherited intuitive myopia. More genome detectives should engage with the historians of life in an effort to connect the dots and enter the quest for the historical outlines of the elephant, rich and strange. ■

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# Wiring up quantum systems

R. J. Schoelkopf and S. M. Girvin

**The emerging field of circuit quantum electrodynamics could pave the way for the design of practical quantum computers.**


In the past two decades, scientists and engineers in a variety of disciplines have been excited by the idea of quantum information processing[1], in which a computation is carried out by controlling a complex collection of quantum objects. This idea seeks to combine two of the greatest advances in science and technology of the twentieth century.

The first breakthrough is the development of quantum mechanics, with its sometimes strange and counterintuitive rules that hold sway in the domain of atoms and single particles. The second is the technological revolution that followed the invention of the integrated circuit and the advent of powerful digital computers, which gave rise to the current information age. Surprisingly, the seemingly bizarre quantum-mechanical ideas of superposition and entanglement are expected to lead to a kind of natural parallel processing during computations. The unlikely marriage of these two revolutions could lead to incredible advances in computational power, at least for certain special problems.

Unfortunately, the practical challenges to making a quantum information device are daunting. To build a quantum computer, the classical bits that store information in an ordinary computer must first be replaced with quantum bits (qubits). These qubits can be composed of any quantum system with two distinct states (0 and 1), but they have the special property that they can be placed into quantum superpositions, existing in both states at once. A computation then proceeds by combining manipulations of the superpositions in single qubits (one-bit operations) and controlled interactions of multiple qubits (the quantum equivalent of logic gates). But to truly exceed the capabilities of conventional computers, the quantum engineer must acquire extremely precise control over the quantum domain, prevent any unknown evolution that affects the quantum states (decoherence), and amass many thousands of qubits. Moreover, these qubits must then be 'wired up' in complex and prescribed arrangements, so that they can interact and communicate their quantum information back and forth during the computation.

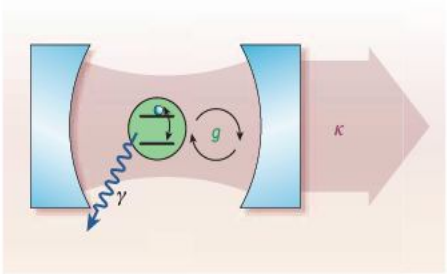



**Figure 1 | Cavity quantum electrodynamics.** Schematic representation of a cavity quantum electrodynamics (QED) system, consisting of an atom with two energy levels interacting with a single photon mode (pink) trapped by mirrors (blue) to form a cavity. The blue dot is an electron occupying one of the energy levels. The strong coupling regime is reached when the interaction rate of the atom and a single photon ($g$) is larger than the dissipation arising from the loss of photons (at rate $\kappa$) or from emission from the atom into other modes at rate $\gamma$; in other words, when $g >> \kappa, \gamma$.

Many different physical implementations of quantum information processors are being pursued today. Some systems comprise 'natural' candidates, such as single atoms, ions or spins, for which the manipulation of quantum states has a long history and is routine in many laboratories. Others are based on artificial systems in the solid state, such as quantum dots or superconducting circuits. These latter candidates have a certain appeal as they can be designed and fabricated using techniques borrowed from conventional electronics.

Before making a quantum information processor from solid-state systems such as superconducting circuits, two basic questions must be addressed. First, can the qubits be made from sufficiently 'atom-like' circuit elements, in which the macroscopic variables such as current and voltage can exist in controllable superpositions of distinct quantum states? And second, can we connect these qubits together in the required manner, perhaps using familiar electrical means such as actual wires, but keeping in mind that any information transported must remain in its intrinsically quantum form and exchanged as individual quanta?

The answer to the first question, originally posed[2] to test the applicability of quantum mechanics for macroscopic objects, is now at least a qualified 'yes'. Pioneering work in the 1980s on simple superconducting circuits incorporating a Josephson junction[3] (see Box 1) showed that macroscopic variables such as voltages could indeed exhibit quantum behaviour. Further work established that junctions could be considered as 'atoms with wires', which display energy-level quantization[4] and interact strongly with the electromagnetic environment[5,6]. It was not until the end of the 1990s, however, that the first evidence for coherent superpositions[7] and time-domain control of the quantum state[8] in a superconducting qubit was demonstrated.

The past decade has seen rapid progress in this field. Several different 'flavours' of superconducting qubit[9] (see Box 1) have now been demonstrated, and two qubits have been coupled to demonstrate the entanglement between them[10] and to perform simple quantum logic operations[11]. The current state-of-the-art allows for superposition states that survive for several microseconds, long enough for hundreds of operations on a single qubit. With improvements in superconducting qubit design, as well as in the materials and methods used for fabricating circuits, the lifetime of the stored quantum information may be further increased and the precision of qubit control and measurement enhanced.

But how can we address the second question and realize the quantum connections between qubits? For communicating quantum information between real atoms, optical photons are natural candidates[12]. They have many advantages, including rapid propagation and the ability to be guided on optical fibres for many kilometres without being lost. Superconducting qubits also interact electromagnetically, but because of their much smaller energy-level separations, the 'photons' they best couple with lie in the microwave range of the spectrum (frequencies of 3–30 GHz, or wavelengths of 1–10 cm). Several authors[13–22] have speculated that such microwave photons could be a route to connecting qubits, and recent experiments[23–30] have demonstrated qubit–photon couplings in superconducting circuits. This approach is similar to the branch of atomic physics known as cavity quantum electrodynamics (cavity QED), which studies the interaction of photons

and atoms at the quantum level. The new field, dubbed 'circuit QED', offers a tentative 'yes' to the second key question about whether we can create quantum devices with interconnected qubits.

Here we begin by discussing the physics of cavity QED with real atoms, and then introduce the analogous circuit QED system, in which microwave photons are coupled to superconducting qubits acting as artificial atoms. As we will see, the tight confinement of microwaves on a chip naturally leads to extremely strong 'atom–photon' coupling, offering new possibilities for fundamental experiments on the light–matter interaction and interacting quantum systems. After reviewing various experiments in circuit QED that have been performed to date, we will point out some of the outstanding issues and future directions for this rapidly progressing field.

## An atom meets a photon

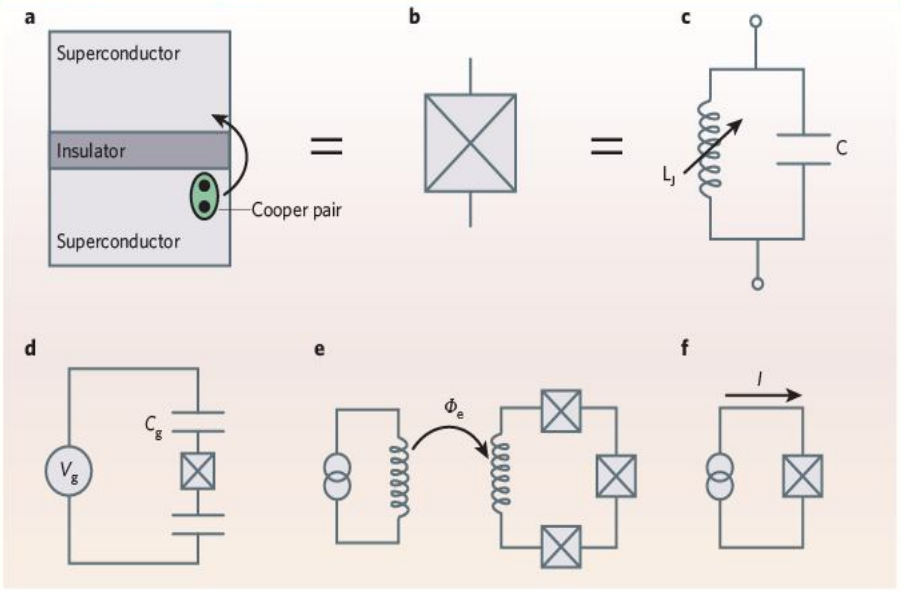
Cavity QED[31–33] is the physicist's prototype system for studying the interaction of light and matter at the quantum level. At its simplest, it consists of a single atom with just two relevant quantum states, coupled to a single mode of the electromagnetic field, defined for example by a pair of mirrors (Fig. 1). A photon in the cavity, bouncing back and forth between the mirrors, can be absorbed by the atom; conversely, if the atom is excited, it can decay by emitting a photon into the cavity. The rate of this atom–light interaction ($g$) is proportional both to the dipole moment of the atom and to the electric field of the photon at the atom's location (see Box 2, overleaf). Unfortunately, in any real system, other undesired processes can take place, such as a loss of photons from the cavity (at rate $\kappa$) resulting from imperfect mirrors, or the decay of the atom (at rate $\gamma$) into other channels.

The 'strong coupling regime' of cavity QED is obtained when the rate of absorption or emission of a single photon by the atom is more rapid than any of the rates of loss ($g >> \kappa,\gamma$). In this case, an excited atom in an initially empty cavity will emit one (and only one) photon, which can then be trapped and reabsorbed again (at rate $2g$), a phenomenon known as vacuum Rabi oscillations. The presence of the cavity has made the spontaneous emission from the atom, usually an irreversible process, into a coherent and reversible oscillation. Entering this regime dramatically reveals the quantum nature of the electromagnetic field, allows the experimenter to make and measure non-classical states of light, and makes experiments in nonlinear optics possible at the level of a single photon. In the language of quantum computation, strong coupling means that quantum information can be exchanged back and forth between the atom and the photon many times before it is lost for ever.

The challenge for realizing strong-coupling cavity QED is to maximize the vacuum Rabi frequency (see Box 2) while simultaneously minimizing the decay ($\kappa,\gamma$). Obviously, it helps to have an atom that is a strong emitter, with a large dipole moment. To enhance the coupling further, the photon's energy should be confined in the smallest cavity possible, so that the corresponding electric fields are spread over the minimum volume. Equivalently, one can imagine that the mirrors act to reflect the photon past the atom repeatedly, giving many chances for the interaction to take place. At the same time, the atom should be as decoupled as possible from other influences, and the cavity loss kept small. A further difficulty is the placement and trapping of a single atom at the desired location in the cavity.

Despite the obvious technical challenges, there are several examples of strong-coupling cavity QED using real atoms. For optical photons trapped between mirrors, the vacuum Rabi splitting, which indicates strong coupling, was first observed back in 1992 (ref. 34). Another approach[35–38] uses 'Rydberg atoms', which are highly excited atomic states that have very large

### Box 1 | Superconducting qubits




In a superconductor well below its transition temperature, electrons are strongly bound together in 'Cooper pairs', enabling electrical signals to propagate with very low dissipation. Superconducting qubits are based on Josephson junctions, which are made by two pieces of superconductor separated by an insulating layer thin enough to allow tunnelling of Cooper pairs (**a**). A Josephson junction is usually denoted in circuit diagrams as a box with a cross (**b**).

A dissipation-free supercurrent can then flow through the junction, which turns out to be equivalent to a nonlinear inductor[10]. The physical realization of the junction (**c**), with two electrodes separated by an insulator, makes an LC circuit (a capacitor, C, and inductor, $L_J$, in parallel), which is the electrical equivalent of a harmonic oscillator.

The Josephson junction is a very special oscillator, however, as it combines nonlinearity with low dissipation. The nonlinearity means that the energy levels can be anharmonic (not regularly spaced) and, with the right circuit configuration, two low-lying states can be obtained that are sufficiently separated from the others so that the junction can be treated as a quantum two-level system, or a qubit. The typical energy separation is large enough to probe at millikelvin temperatures in cryogenic refrigerators.

Three main 'flavours' of superconducting qubit have been used, classified according to the variables by which they are controlled and excited. The simplest qubit is the charge qubit (**d**), or Cooper-pair box, which consists of an isolated Josephson junction placed between the plates of a capacitor. Applying a voltage ($V_g$) to the capacitor ($C_g$) induces a charge difference between the two sides of the junction. Alternatively, one can say that the qubit responds to electric fields.

The second type is the flux qubit (**e**), consisting of a superconducting ring interrupted by one or more (often three, as here) Josephson junctions. A current through an external inductor generates a magnetic flux ($\Phi_e$) threading the loop, which induces clockwise or anticlockwise circulating supercurrents. This qubit couples to magnetic fields.

The third type of qubit is the phase qubit (**f**), consisting of a single Josephson junction connected to a current source. Current ($I$) flowing through the junction alters the phase difference between the two sides of the junction.

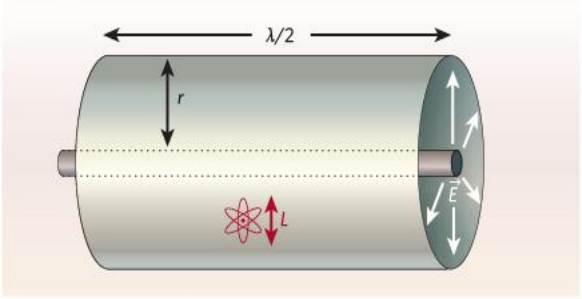
All three flavours of qubit have been used successfully, and the ability to make and control superpositions has been demonstrated. The typical lifetime of a quantum superposition is on the order of a microsecond, allowing hundreds of single-qubit operations. Experiments with two and three qubits coupled to each other, including the generation of entangled states and the operation of a conditional-NOT logic gate, have also been performed. **R.J.S. & S.M.G.**

**Box 2 | The fine-structure limit for cavity QED**

A simple calculation[31,53] shows that the coupling strength (the vacuum Rabi frequency) of an atom and a photon in cavity quantum electrodynamics (QED) has an upper limit given by fundamental constants. A photon excites the atom by moving one of its electrons into a larger orbit; the 'dipole moment' ($d = eL$, with units of charge × distance, where $e$ is the electron charge) is a measure of the size of the atom, and also determines how strongly the atom interacts with a given electric field. The vacuum Rabi frequency is thus given by $g = dE_0/\hbar$, where $E_0$ is the root-mean-square electric field at the location of the atom due to vacuum fluctuations ($\hbar$ is the Planck constant). The vacuum fluctuations exist in both electric and magnetic fields, and have an amplitude equal to that due to half a photon. A simple estimate of this electric field can be obtained by adding up the density of energy ($\varepsilon_0 E^2/2$) stored in the electric fields, which must be equal to half the energy of a photon (remembering that half of this energy is also stored in magnetic fields):

$$\frac{\hbar\omega}{4} = \frac{\varepsilon_0}{2}\int E^2 dV = \frac{\varepsilon_0}{2} E_0^2 V$$

where $\varepsilon_0$ is the permittivity of free space, $\omega$ is the transition frequency of the atom/cavity and $V$ is the volume of the cavity. Thus, the field strength increases as the volume of the cavity is decreased and the photon is more tightly confined. However, a typical three-dimensional cavity used with real atoms will have a volume that is many cubic wavelengths.




In circuit QED, we can use a one-dimensional transmission-line cavity, like the simple coaxial geometry shown here, which must be half a wavelength long but can be much smaller in the transverse direction, and have a volume, $V = \pi r^2 \lambda/2$, much less than a cubic wavelength. This leads to a greatly enhanced field strength:

$$E_0 = \frac{1}{r}\sqrt{\frac{\hbar\omega^2}{2\pi^2\varepsilon_0 c}}$$

where we have used the fact that the wavelength $\lambda = 2\pi c/\omega$ and $c$ is the speed of light. Multiplying this field strength by the dipole moment, we can express the vacuum Rabi frequency in dimensionless units:

$$\frac{g}{\omega} = \left(\frac{L}{r}\right)\sqrt{\frac{e^2}{2\pi^2\varepsilon_0\hbar c}} = \left(\frac{L}{r}\right)\sqrt{\frac{2\alpha}{\pi}}$$

we find that the dimensionless combination of the fundamental physical constants of electromagnetism, the fine-structure constant $\alpha = e^2/4\pi\varepsilon_0\hbar c \sim 1/137$, has appeared. The best situation is to arrange a cavity whose transverse size is small enough that the atom completely fills the transverse dimension ($L/r \sim 1$), and then the coupling can be several per cent. In comparison, because the three-dimensional cavities in either optical or microwave cavities have bigger sizes and the real atoms used have smaller dipole moments, the largest couplings possible so far have much smaller $g/\omega$, on the order of $10^{-6}$. The large interactions achievable in the one-dimensional cavities of circuit QED make it easier to attain the strong-coupling limit. **R.J.S. & S.M.G.**

dipole moments and low energy transitions at ~50 GHz. In this case, the photons are in the microwave domain and the cavity consists of a superconducting metallic box a few wavelengths (several centimetres) across. Other efforts have focused on strong coupling with semiconductor quantum dots as the emitters; these have the potential advantage of emitting at infrared wavelengths close to those used for telecommunication[39–41]. The coupling of internal states of an atomic ion to its motion in a trap[42,43] can also be understood as a realization of strong-coupling cavity QED, because it contains the same essential ingredients of a two-level system (the ion) interacting with a harmonic oscillator (the quantized motion of the ion, or phonons).

Many beautiful experiments have been done in the past two decades using these strongly coupled cavity QED systems, performing textbook demonstrations of fundamental quantum phenomena such as decoherence and entanglement. A spectacular recent achievement is the ability to perform quantum 'non-demolition' experiments, in which photons in a microwave cavity can be monitored without destroying them, revealing the progressive collapse of the wavefunction under successive measurements[44,45]. Other efforts have developed quantum 'applications', such as the creation of sources of non-classical light and single photons on demand[46], or the detection of single atoms and the cooling and manipulation of their motion.

The ability to control the interactions of atoms and single photons in a quantum-mechanical way has intriguing implications for quantum computation and communication. Photons in a cavity[47,48], or phonons in an ion trap[49], can be used to generate entanglement and make a 'quantum bus' to communicate quantum information between multiple atoms. But the technical difficulties of achieving sufficiently strong coupling, trapping many atoms, and then individually addressing and controlling them, make it difficult at present to build large-scale quantum systems.

## Quantum optics on a chip

Circuit QED is a more recent attempt to bring about strong coupling within an integrated superconducting circuit[22]. This approach offers the prospect of reaching an upper limit for strong coupling. Josephson-junction qubits (see Box 1) can play the role of the atom or the matter component, but how can we trap a photon on a chip? At the microwave frequencies emitted by superconducting qubits, photons can exist in three-dimensional form as standing waves in a metallic box a few wavelengths across, like those used in the Rydberg-atom experiments. In the world of electrical circuits, however, photons can also be understood as the quantized excitations of any electromagnetic resonator, including the simple combination of an inductor and a capacitor[50]. Such an electrical oscillator can in principle be much smaller than a wavelength in all dimensions, so that the 'photons' are confined very tightly indeed, and are effectively zero-dimensional. Another possibility is that photons are confined in one dimension and travel along a transmission line, not unlike the coaxial cable used for TV transmission. A key realization[22] was that strong coupling might be achieved as a result of the tight transverse confinement, while still having a long 'wire' that can transport signals from place to place.

An implementation of circuit QED using a transmission-line resonator whose electric fields are coupled to a superconducting charge qubit is shown in Figure 2a. A central superconducting wire running between two ground planes defines the transmission line. Gaps in the wire, placed an integer number of half-wavelengths (a few centimetres) apart, are the 'mirrors' used to form a cavity, which is the microwave version of the Fabry–Pérot geometry used in optics. The size and shape of the gaps controls the rate at which photons enter and leave the cavity, and the entire structure can be made using conventional microelectronic fabrication techniques. Such superconducting transmission lines have been extensively studied in the past. But recent experiments at temperatures close to absolute zero, where they are used as detectors for astrophysics[51,52], have shown that photons can make up to a million bounces before being lost (the 'cavity quality factor', $Q$, is $10^6$). This means that the losses are remarkably low — a gigahertz photon travels back and forth a total distance of 10 kilometres before being lost.

The qubit, an isolated Josephson junction, is placed between the wire and the ground planes, at or near an antinode of the standing wave of the voltage on the line, so it couples to the electrical fields of the transmission line. Exciting the qubit corresponds to transporting one or a few pairs of bound electrons (known as Cooper pairs) from one electrode of the junction to the other. This means that the

dipole moment of this artificial atom is very large, often more than four orders of magnitude greater than the typical value for an electronic transition of a real atom. Because the qubit's size and shape are adjustable, the dipole coupling can also be engineered by having the atom essentially fill the transverse dimension of the cavity, which means that the vacuum Rabi frequency (expressed as a fraction of the photon frequency) approaches a maximum value[53] of a few per cent, set by the fine-structure constant (see Box 2). In comparison, the best values obtained so far using real atoms in either optical or microwave cavities are much smaller, of the order of one part in $10^6$. The very large interactions achievable in circuit QED make it easier to attain the strong coupling limit of cavity QED. Another advantage of circuit QED is that it avoids the difficulties of cooling and trapping the atom, as the qubit can be fabricated at precisely the desired location inside the cavity.

Several experiments with superconducting qubits in the past few years have accessed the regime of strong coupling, and have recapitulated many classic results from quantum optics. Strong coupling with circuit QED was first achieved in 2004 (refs 23, 24), and a device like that shown in Figure 2b has been used[23] to observe vacuum Rabi splitting in a solid-state, artificial system. When transmission through the cavity was measured when the qubit was tuned into resonance, two separate peaks (the vacuum Rabi splitting) could be resolved (see Fig. 3a, overleaf), corresponding to coherent superpositions of a single photon in the transmission line and a single excitation of the qubit. A more recent experiment[54] with an optimized qubit now approaches the fine-structure limit, with a dimensionless coupling strength of about 2.5%, yielding the large splitting shown in Figure 3b. Other experiments have observed vacuum Rabi oscillations in the time domain[25] and demonstrated a maser based on a single artificial atom[30].

Circuit QED has also been used for quantum communication and coupling between qubits. A source of non-classical microwaves has been demonstrated, for example, in which single photons are produced on demand[27]. This experiment also showed that the quantum information contained in a superposition state of a qubit could be mapped onto the photon state, demonstrating the conversion between a standing and a flying qubit, a milestone for quantum computation. Finally, a cavity has been used to realize a solid-state quantum bus, with a quantum state being transferred from one qubit to another using a microwave photon as the intermediary. This last achievement was made simultaneously in experiments with phase qubits[29] and charge qubits[28]. Taken together, these experiments indicate that communication between small prototype systems of several qubits, wired together with photons and cavities, is possible. The combination of techniques and concepts from quantum optics, in conjunction with the technology for superconducting quantum circuits, is likely to lead to continued rapid progress.

The combination of circuit QED and experimental advances with superconducting circuits raises many interesting questions, and next we shall discuss some possible themes and areas for future work.

## New regimes of quantum optics

As mentioned above, the relative coupling strength in circuit QED is many orders of magnitude greater than in the better-known versions of cavity QED with real atoms. This means that less-familiar, higher-order effects can have a noticeable influence. One example is the dispersive, or off-resonant, case, in which the qubit and the photon interact without the photon being absorbed. In the 'strong dispersive regime' in circuit QED[26], this interaction, although roughly ten times smaller than the resonant case, is still larger than all sources of decoherence, a situation that has been accessed in only a few experiments with Rydberg atoms[44,45]. Circuit QED couplings can approach the limit where multiphoton effects, which are usually rare, play an important role. Other new phenomena include optical bistability of the cavity, in which the presence of a single atom makes the cavity oscillations strongly anharmonic, and causes the entanglement of multi-photon states. It is also possible to engineer strong photon–photon nonlinearities, based for example on the simultaneous interaction of two cavities with a single qubit.

What is the real limit on the strength of coupling? It should be possible to push coupling strengths beyond the fine-structure limit discussed above for electric fields. For instance, if the current in a transmission line is passed directly through a Josephson junction[53], the relative coupling can be larger than unity ($g > \omega$, where $\omega$ is the transmission frequency of the atom/cavity), so the photon and the qubit cease to be separate entities and the coupling cannot be considered as a perturbation. All these investigations could add significantly to the body of knowledge on the light–matter interaction already gleaned from cavity QED.

## What are the limits of coherence?

Perhaps the greatest outstanding problem with all solid-state implementations of quantum systems is how to minimize decoherence, the inevitable loss of quantum information owing to coupling to undesired degrees of freedom, and secure enough time to allow complex manipulations. In their roughly 10 years of existence, the coherence time of superconducting qubits has increased by a factor of almost 1,000 (from just nanoseconds to a few microseconds), but further improvements will

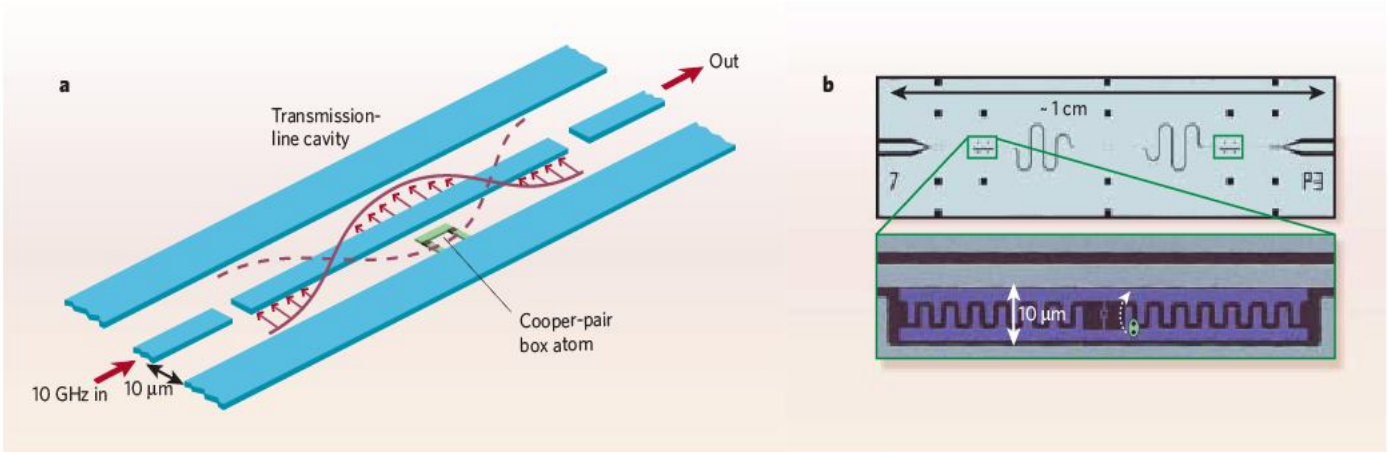



**Figure 2 | Circuit QED devices. a**, Schematic representation (adapted from ref. 22) of the circuit analogue of cavity quantum electrodynamics (QED), where a superconducting qubit (green) interacts with the electric fields (pink) in a transmission line (blue), consisting of a central conductor and two ground planes on either side. The cavity is defined by two gaps (the mirrors) separated by about a wavelength. The cavity and qubit are measured by sending microwave signals down the cable on one side of the cavity and collecting the transmitted microwaves on the output side. **b**, Micrograph of an actual circuit QED device that achieves the strong-coupling limit. It consists of a superconducting niobium transmission line on a sapphire substrate with two qubits (green boxes) on either side. The inset shows one of the superconducting Cooper-pair box charge qubits located at the ends of the cavity where the electric fields are maximal. The qubit has two aluminium 'islands' connected by a small Josephson junction. Changing the state of the qubit corresponds to moving a pair of electrons from the bottom to top (shown schematically).

be necessary. It is not yet clear whether materials, circuit designs or other, unknown factors will ultimately be the limiting factor.

Three-dimensional dielectric[55,56] and superconducting microwave resonators[57] that can store photons for about a second or more already exist. But for the miniaturized, on-chip cavities used for circuit QED, demonstrated photon lifetimes are only about ten times longer than those of a qubit, perhaps tens of microseconds. Because superconducting qubits are actually rather similar to electrical resonators (with the extra ingredient of nonlinearity provided by a Josephson junction), making the lifetime of an on-chip, linear cavity effectively infinite can be viewed as a necessary, but not sufficient, step for making truly robust qubits. Indeed, this quest may teach us how to make better junctions and qubits[58]. If cavity lifetimes continue to exceed those of qubits, they might serve a useful role[29] as a 'quantum memory', where quantum information could be stored as photon superpositions. Because cavities can inherit a nonlinearity from coupling with a qubit, it may be useful to ask what the optimal amount of nonlinearity should be, and to imagine 'photonic qubits' in which energy is shared between linear and nonlinear elements in order to optimize coherence.

## Wiring up elemental quantum objects

We have so far confined ourselves to discussing the circuit QED interaction of superconducting qubits. There are, however, a large variety of elemental quantum objects with microwave transitions, which could in principle be coupled via transmission lines (Fig. 4). Qubits made from fundamental systems such as atoms or spins offer certain advantages, including perfect reproducibility (identical atoms have identical spectra) and longer coherence times, although they can be more difficult to integrate together. These include electric-dipole-coupled systems such as atoms and molecules, or magnetic dipoles such as nuclear and electron spins, which each have their own advantages and disadvantages. They will all interact with the electric or magnetic fields of a photon if placed appropriately inside a cavity, but some will interact more strongly, and others will tend to have longer coherence times. Several approaches for building 'hybrid' quantum systems with both macroscopic, artificial components and microscopic, individual particle elements have been proposed recently[59–61].

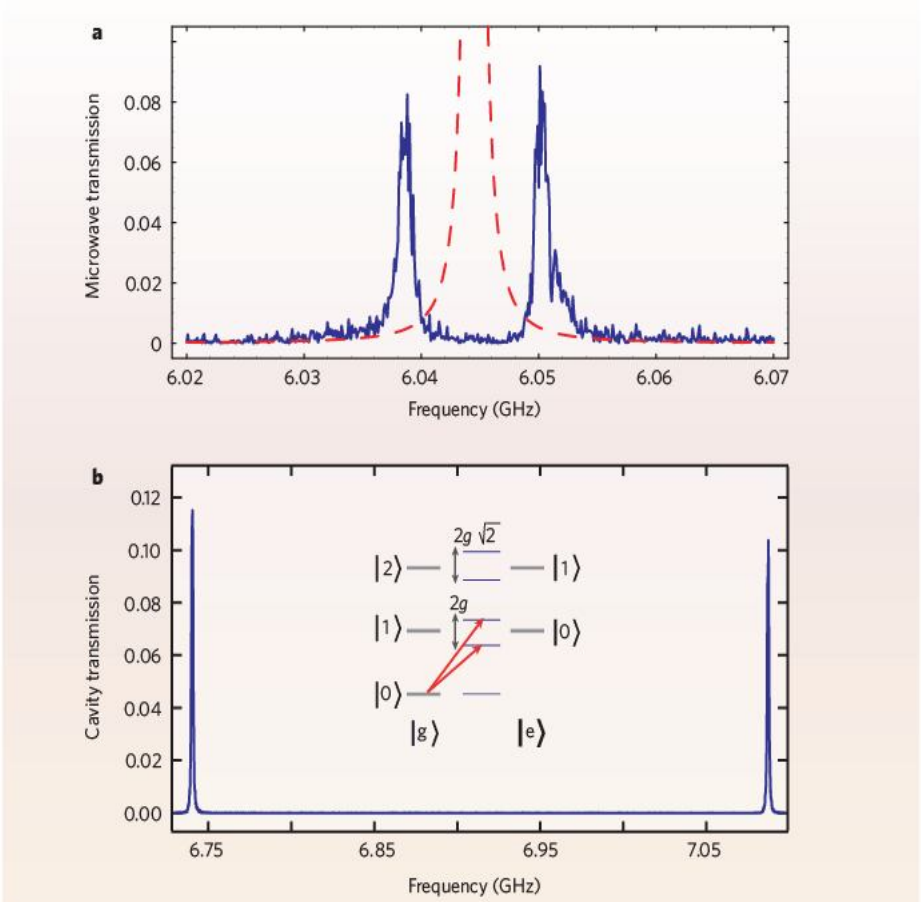



**Figure 3 | Vacuum Rabi splitting.** Observation of strong coupling and the fine-structure limit in a circuit. **a**, Measurement of the microwave transmission of a cavity like that in Fig. 2b (adapted from ref. 23). The appearance of two peaks in the transmission, as a result of vacuum Rabi splitting, indicates strong coupling. Without the qubit, a single transmission peak (dashed line) is observed. With the qubit tuned to match the cavity frequency, the qubit–cavity interaction mixes together the photon and qubit states, and the new eigenstates of the system are coherent superpositions that are symmetric and antisymmetric combinations of atom and photon. The decay rates of these half-atom/half-photon superposition states is the average of the photon and atom decay rate, $(g+\kappa)/2$. Strong coupling is observed by starting with the system in its lowest energy state (with no photons and the atom in the ground state) and measuring the presence of two peaks separated by $2g \sim 12$ MHz about the original cavity resonance. This splitting of the cavity resonance is akin to observing vacuum Rabi oscillations in the frequency domain. **b**, A more recent experimental result, showing a separation of the vacuum Rabi peaks by about $2g/2\pi = 350$ MHz, in which $g/\omega \sim 2.5\%$; the cavity decay rate is $\kappa/2\pi \sim 800$ kHz and the qubit decay rate is $\gamma/2\pi \sim 200$ kHz. This experiment approaches the fine-structure limit (see Box 2) for the maximal value of an electric dipole coupling.

What would make the most ideal 'atom' in a circuit QED or a quantum device? There are various trade-offs, which can be viewed by arranging the systems in a rough hierarchy based on the 'size', or transition moment. In general terms, the larger the size, the higher the vacuum Rabi frequency, and the more rapidly the qubits can communicate via the cavity. But the coherence times of these systems tend to vary inversely, and what counts is the number of operations possible, which is essentially the ratio of coupling and decoherence rates. At one end of the spectrum are Rydberg atoms and superconducting qubits, which have micrometre-scale electric dipoles that can match the size of a typical superconducting cavity and approach the fine-structure limit with vacuum Rabi frequencies of hundreds of megahertz, although these have coherence times on the order of microseconds[9] to milliseconds[60]. In the middle are polar molecules, which are small compared with the cavity and would have correspondingly slower coupling rates, but their coherence times could be more than 1,000 times longer. At the other extreme are spins, which can have lifetimes of seconds but have coupling rates of around a hertz, which is probably too small to be practical. In many cases, ensembles of particles could be used to increase the coupling strength, but at the expense of losing nonlinearity.

Experimental efforts with these hybrid systems are now under way in several laboratories[62,63]. Another approach to communicating quantum information around a chip is to actually transport the qubits themselves. This is already being done for trapped ions based on microfabricated traps[62,64]. As well as being an approach to engineering quantum processors, all this work may lead to new ways to cool and manipulate quantum objects, and perhaps even to new kinds of spectroscopy and precision measurements. Manipulating rotational or hyperfine microwave transitions in atoms and molecules can influence the electronic transitions at optical wavelengths, which are accessible simultaneously. This might eventually lead to the possibility of transferring quantum information from a chip to an optical fibre. Such a quantum interconnect is a highly desirable feature for quantum repeaters and communication.

## Making a complex quantum state

Through its ability to use photons to communicate between several qubits, circuit QED

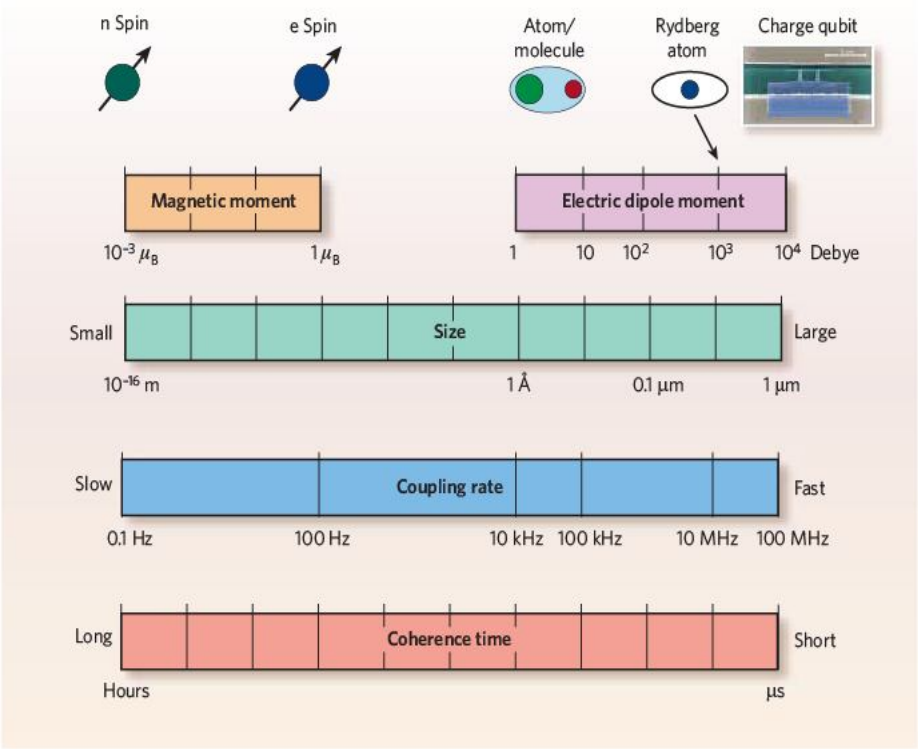



**Figure 4 | Wiring up quantum systems.** Besides a Cooper-pair box, many other quantum systems have microwave transitions that could be coupled to photons in a transmission line. What is important for quantum computation is the number of operations that can be performed, so the longer lifetimes of smaller particles can partly offset the weaker couplings. For a line with transverse dimensions on the order of a micrometre, the electrical field strengths for a single 5-GHz photon are $E_0 \sim 1.4\ \mathrm{V\,m^{-1}}$ (see Box 2), and the corresponding vacuum Rabi coupling rate is $g/2\pi \sim 10$ kHz for a 1-debye dipole moment. Magnetic dipoles such as spins could also couple to the corresponding magnetic fields, $B_0 \sim 0.1$ milligauss, with vacuum Rabi rates of about $g/2\pi \sim 100$ Hz per Bohr magneton. Quantum systems can be compared according to their electric or magnetic dipole moments (or the magnitude of the emitter strength, top bars), the required transverse size of a cavity to reach maximal coupling (second bar), their coupling rates (third bar) to a technologically feasible (1 μm) cavity, and the expected lifetimes of coherent superpositions (bottom bar). Which quantum system is optimal depends on many details, including the ease of trapping or fabricating in a cavity, and on the many factors in the qubit environment that can affect the coherence times.

may help to bring about more complex quantum systems with superconducting circuits. The next step could be to demonstrate multiparticle entanglement and develop simple schemes for quantum error correction.

Some of the most beautiful investigations of quantum optics have shown that the most counterintuitive properties of quantum mechanics, such as entanglement, nonlocality and the measurement problem, are real. What might we learn by extending these tests to engineered, macroscopic systems?

To build even a small quantum information device, we will need unprecedented control over matter at the quantum level. Is the often-cited factoring of large numbers the only, or indeed the most interesting, way to exploit such an amazing capability? It is possible that quantum computers of this sort will simply prove too difficult to build. So finding short-term applications for smaller quantum machines that justify the effort may be crucial to the future of these endeavours.

A final point is that, during a large-scale quantum computation, the device will need to occupy devilishly complex quantum states, which we have little experience with so far. We may find that there is a fundamental principle, which we haven't discovered yet, that prevents their existence. Such a possibility might even mark the end of the road for quantum computing — but provide a new beginning for basic science. ■

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REVIEWS

# Towards a molecular understanding of shape selectivity

Berend Smit[1,2,3]† & Theo L. M. Maesen[4]†

**Shape selectivity is a simple concept: the transformation of reactants into products depends on how the processed molecules fit the active site of the catalyst. Nature makes abundant use of this concept, in that enzymes usually process only very few molecules, which fit their active sites. Industry has also exploited shape selectivity in zeolite catalysis for almost 50 years, yet our mechanistic understanding remains rather limited. Here we review shape selectivity in zeolite catalysis, and argue that a simple thermodynamic analysis of the molecules adsorbed inside the zeolite pores can explain which products form and guide the identification of zeolite structures that are particularly suitable for desired catalytic applications.**

Zeolites are microporous mineral materials that have found wide use in industry since the late 1950s, with one of their most important applications being chemical catalysis. They are particularly important as cracking catalysts in oil refining. One of their defining features—apart from being solid catalysts that are easy to recycle—is that the shape, or topology, of the internal pore structure of a zeolite can strongly affect the selectivity with which particular product molecules are formed in chemical transformations catalysed by the zeolite. Here, we will argue that this shape selectivity can be explained by very simple thermodynamic analyses that consider the impact of zeolite topology on the free energy landscape; that is, on the free energies of formation of the various molecules involved in the catalysed reactions.

The analyses presented here are simple and straightforward, yet have become feasible only relatively recently as advances in molecular simulation techniques have started to provide access to the thermodynamic data underpinning them. After a short introduction of zeolites and their use as catalysts, we will therefore also briefly outline the developments in simulation capabilities that give access to the thermodynamic information crucial for our understanding of zeolite catalysis. We then show how the free-energy-landscape approach can elucidate the molecular-level mechanism(s), giving rise to shape selectivity in a number of simple yet industrially important processes. We conclude this review by outlining the crucial issues that need to be addressed to take the free-energy-landscape approach to the next stage, where the combined use of simulations and thermodynamic analysis might have profound implications for how we screen and develop zeolite-based catalysts.

## Zeolites as industrial catalysts

Zeolites are crystalline aluminosilicates with a three-dimensional framework that consists of nanometre-sized channels and cages and imparts high porosity and a large surface area to the material. The basic structural unit of all zeolite frameworks consists of a silicon or aluminium atom tetrahedrally coordinated to four oxygen atoms. Any zeolite built of silica and oxygen only is neutral, but replacing $Si^{4+}$ by $Al^{3+}$ creates a negative charge on the framework. All such framework charges are neutralized by cations that reside inside the zeolite pores, where they can move freely and be exchanged against other cations. When protons neutralize framework charges, they constitute acid sites that can catalyse the two types of reaction important in all oil refining: the isomerization and the cracking of hydrocarbons[1]. Depending on the topology of the zeolite used and the selectivity it imparts, isomerization and cracking reactions form desired products by converting simple *n*-alkanes into various branched isomers and cleaving large hydrocarbons into smaller ones, respectively[2]. The majority of the constituents of many everyday substances, from gasoline to a plastic PET bottle, will thus have seen the inside of a zeolite catalyst and experienced the effect of shape selectivity[3]. Yet, despite this enormous economic importance of shape selectivity, we have only recently gained the insights needed to fully understand the molecular mechanisms that give rise to it.

This review focuses on hydroconversion reactions that proceed in the presence of excess hydrogen gas because they are relatively well understood and exemplify the recent improvements in understanding of shape selectivity. In hydroconversion reactions the acid sites inside the zeolite pores catalyse two competing reactions upon hydrocarbon exposure: hydroisomerization and hydrocracking. The hydroisomerization reactions convert linear hydrocarbons (*n*-alkanes) into branched isomers; these can then be converted further through transfer of the branch along the molecular backbone, or through a second hydroisomerization reaction to form a di-branched isomer. Hydrocracking reactions break a hydrocarbon reactant into two smaller molecules, and proceed particularly easily if hydroisomerization has formed a so-called hydrocracking precursor: a molecule with two branches that are attached to the same carbon atom or to neighbouring carbon atoms. The detailed mechanism of the elementary hydrocarbon hydroconversion steps is well understood[4]. However, the large number of possible reactions and the fact that many molecules may act as intermediates or end up as products typically results in a complex distribution of product molecules that is not simple to predict.

Figure 1 illustrates hydroisomerization and hydrocracking reactions for a simple starting material (or 'feed') of pure decane. Suppose we carry out a hydrocracking reaction in sulphuric acid or with unstructured (amorphous) aluminosilicates. Because the (gas-phase) free energies of formation of the various decane isomers shown are nearly identical, the product distribution after cracking

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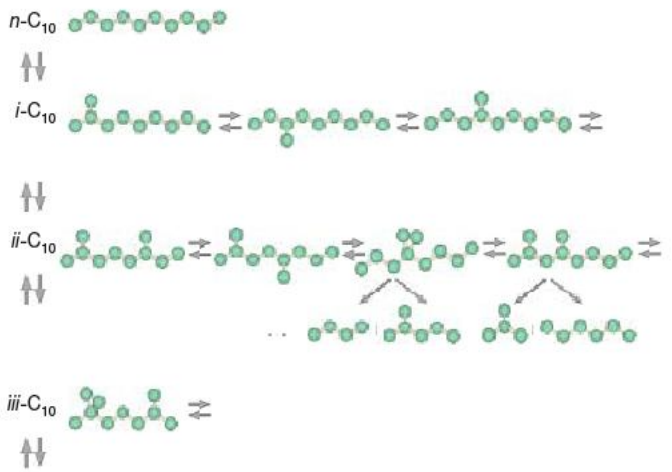



**Figure 1 | Hydroisomerization and hydrocracking of *n*-decane.** The scheme illustrates some of the chemical reactions that can take place inside the pores of a zeolite. These include hydroisomerization reactions that convert *n*-decane (*n*-$C_{10}$) into its mono-branched (*i*-$C_{10}$), di-branched (*ii*-$C_{10}$), tri-branched (*iii*-$C_{10}$)… isomers. A hydrocracking precursor is formed when hydroisomerization yields a molecule with two branches attached to the same or to next-neighbouring carbon atoms. Precursors with two branches hydrocrack relatively easily into a smaller linear and branched alkane. These isomers or their cracking products either continue to react or leave the zeolite as part of the product distribution.

will reflect the probability with which particular cracking precursors form. If all isomers form with equal probability, then simple statistical arguments[5] predict that the highest number of reaction paths lead to hydrocracking precursors that have methyl groups close to the centre of the molecule. As illustrated in Fig. 1, such a molecule will be cleaved or 'cracked' between the two methyl groups. The net result is that the product molecules have a gaussian size distribution centred on $C_5$, that is, on half the length of the feed molecule. Such ideal product distributions simply reflect the statistical probability of forming intermediates and product molecules and are obtained with hydroprocessing catalysts lacking shape selectivity. Zeolite catalysts that give rise to products deviating from the (statistically determined) ideal distribution exhibit shape selectivity. Here we focus on the simplest of cracking reactions involving the metal-catalysed activation of feed molecules through hydrogen ($H_2$) abstraction and the metal-catalysed deactivation of product molecules through hydrogen addition. When this metal functionality is absent (as in catalytic cracking) or defective, then hydrogen subtraction and addition can occur through several different pathways so that the reaction network becomes more complex. In such situations, it becomes commensurately more difficult to identify shape selectivity unambiguously[51].

The original explanation[6] for the shape selectivity associated with zeolite catalysis is simple and intuitive: the pores, or rather the active sites within the pores, exclusively process the molecules that fit inside. Yet in many instances, this picture cannot explain the mixtures of product molecules (the so-called product distributions) obtained in actual zeolite catalysis experiments and new forms of shape selectivity have been discovered (see Box 1)[7,8]. For a more complete understanding of shape selectivity, we need to understand the effect of confinement on the various kinetic and thermodynamic effects that can influence the outcome of a zeolite-catalysed reaction. We show here that this requirement equates, in essence, to understanding the effect of confinement on adsorption and diffusion.

Significant technical advances over the last decades have made it possible to synthesize and characterize well-defined zeolite crystals, and to measure diffusion and adsorption processes accurately in a number of zeolite/hydrocarbon systems. But it is still not possible to obtain reliable experimental thermodynamic and kinetic data on a catalytic system under operating conditions. Here we argue that molecular simulation capabilities can now provide reliable thermodynamic and transport data, and that this capability enables systematic analyses of possible mechanistic explanations for experimentally observed product distributions. Such analyses, in turn, point to a general concept that explains the different types of shape selectivity seen in a wide range of catalytic systems. We show that this general shape selectivity concept not only explains known data and behaviour, it can also serve as a prognostic tool for the simulation-based discovery of those zeolite structures that are best suited for delivering the product distribution desired in a 'real' industrial hydrocarbon processing step.

## Simulating molecules in zeolites

Although recent years have seen much progress in our ability to probe and image single molecules directly, it is at present still impossible to monitor directly how individual molecules move and react inside the pores of a zeolite under operating conditions. But these processes can be simulated on a computer. For such a simulation to deliver realistic results, it needs to use an accurate potential that correctly, and as quantitatively as possible, describes the interactions between the molecules that are present in the zeolite and between those molecules and the zeolite itself. If useful thermodynamic and transport properties are to be extracted, the simulation needs to run long enough for the system to explore the huge number of available configurations; that is, the simulation must 'sample' a sufficient number of different configurations to permit a meaningful statistical description of the results.

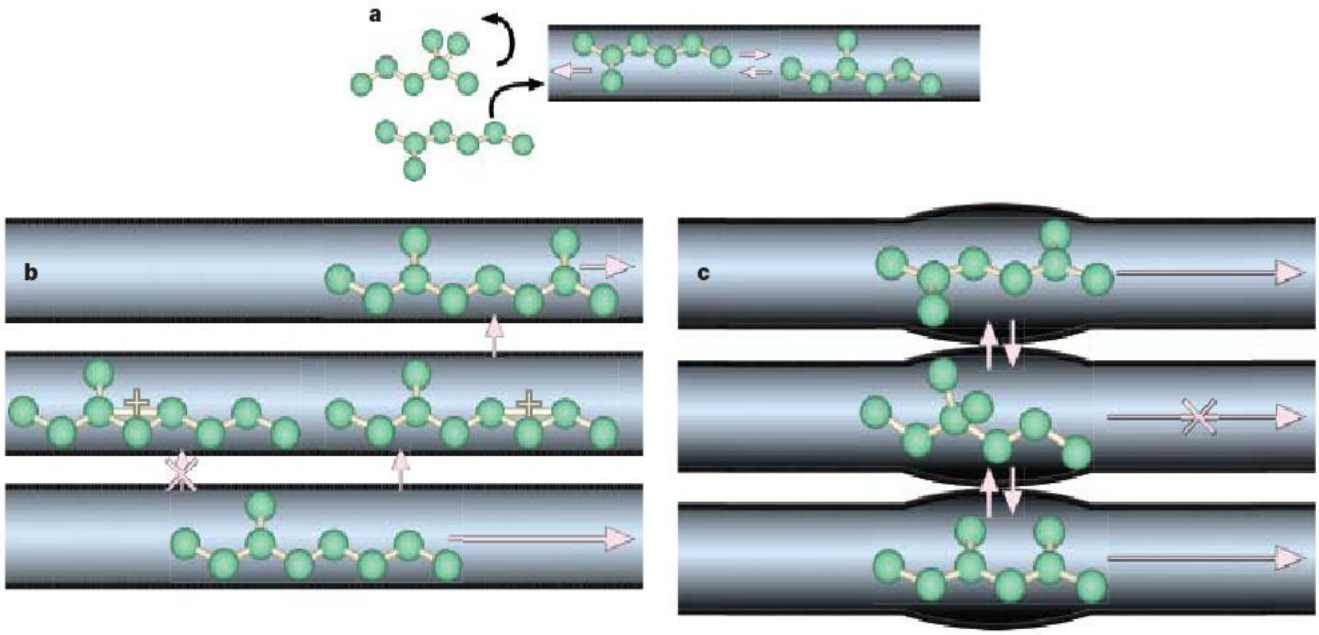
One of the first molecular dynamics simulations of molecules adsorbed in zeolites was published by Thomas and co-workers nearly 20 years ago[9]. Like other early work, it focused on the adsorption thermodynamics and diffusion of small molecules such as methane. But to be pertinent to our understanding of industrial zeolite catalysis, simulations need to investigate not small molecules like methane but long-chain hydrocarbons—an impossible task 20 years ago, simply because simulating long-chain hydrocarbon dynamics in zeolites would have required many millions of years of computer processor time. Their slow dynamics arises from the relatively small diffusion coefficients of long-chain hydrocarbons: they can be orders of magnitude smaller than that of methane, so the molecular dynamics simulations need to be commensurately longer[10] to ensure that the molecules have diffused sufficiently far away from their initial position to generate new and statistically independent configurations and to provide meaningful sampling.

The challenge of how to simulate slowly diffusing molecules can be addressed by considering that such molecules are mostly trapped inside cage-like pore structures or at adsorption sites. Infrequent hopping to a neighbouring cage or adsorption site occurs only after a molecule has overcome a considerable free-energy barrier to diffusion. Such situations can be dealt with by exploiting the stochastic nature of Monte Carlo methods when inserting molecules in a zeolite: random insertions will occasionally occur at positions that correspond to the top of the free-energy barrier to diffusion, and the subsequent evolution of the system can then be simulated. This approach deals successfully with the computational problems caused by large diffusion barriers. But conventional Monte Carlo methods can efficiently insert only simple molecules like methane. In the case of long-chain molecules, billions of configurations would need to be generated to find one in which none of the hydrocarbon atoms overlaps with the zeolite or any of the other molecules included in the system. This difficulty can be overcome with an intelligent growing scheme that locates empty spots in the zeolite and then 'grows' the molecule. The most advanced simulation techniques combine these methods for dealing with diffusion and insertion problems and thus make it possible efficiently to simulate the behaviour of long-chain hydrocarbons in zeolites. One of the most successful techniques is configurational-bias Monte Carlo (CBMC). Depending on the simulation conditions used and the size of the inserted molecule, CBMC

**Box 1 | Different forms of shape selectivity**

The historical definition of shape selectivity is that the product distribution will deviate from the ideal distribution if the formation of some of the molecules in Fig. 1 is inhibited by the constraints on molecular size and shape imposed by the pores. Besides exclusion due to the size of the molecules, the Box 1 figure provides a schematic illustration of the three additional effects that determine whether or not a particular product molecule will be formed with a zeolite catalyst of a given pore shape. In the most general case of reactant shape selectivity (Box 1 Figure a), one has a multi-component feed and only those molecules that are adsorbed by the zeolite and diffuse sufficiently fast to the active site will be converted. But the feed molecules not only need to reach the active site, they also have to be converted and the product molecules then need to diffuse away and finally desorb from the zeolite. The shape of the zeolite may influence each of these steps such that the product distribution changes. If a change in product distribution arises from the actual product formation step, the effect is known as transition state shape selectivity. Such a case is illustrated in Box 1 Figure b, where the shape of the zeolite pore inhibits the formation of a bulky (di-branched) molecule that is too big to fit inside. But transition state shape selectivity can also result in inverse shape selectivity: if the bulky product molecule being formed fits the zeolite pore optimally, it will be stabilized and hence can be formed preferentially over other products. Finally, Box 1 Figure c illustrates product shape selectivity, where diffusion limitations prohibit desorption of product molecules that are too large.

Consideration of these three basic effects underlying shape selectivity can give a good indication of which product molecules are likely to form. But for more reliable—ideally quantitative—predictions, we also need to take into account thermodynamic effects. For example, of all possible products, the molecule(s) with the lowest free energy of formation in the adsorbed phase will be preferentially formed (transition state shape selectivity) and the molecule(s) with the highest free energy of adsorption will preferentially desorb and accumulate in the product slate (product shape selectivity). Similarly, of all the reactants that can fit inside a zeolite catalyst, those with the lowest free energy of adsorption will be preferentially adsorbed and can then undergo reaction (reactant shape selectivity).




**Box 1 Figure | Basic mechanisms giving rise to shape selectivity.** **a**, Reactant shape selectivity: molecules that are too large to enter the zeolite pores cannot reach acid sites for reaction and are therefore not converted into products. **b**, Transition state shape selectivity: molecules (and transition states) that are too large to fit inside a pore do not form. **c**, Product shape selectivity: new molecules are formed in the adsorbed phase, but are too large to desorb as a product.

can be 10 to 40 orders of magnitude more efficient than conventional techniques[11]. Box 2 gives a more detailed description of this method and outlines how it can be used to obtain accurate adsorption isotherms[12], free energies of formation of molecules residing inside zeolite pores, and diffusion coefficients[13,14].

## The free-energy landscape

As just discussed, simulations can provide us with reliable thermodynamic and kinetic data characterizing the adsorption and diffusion of organic molecules within a zeolite. This allows us to illustrate and validate the key concept we wish to present here, the 'free-energy landscape approach' to a molecular understanding of shape selectivity in zeolite catalysis. A central premise of this approach is that by ignoring the detailed chemical characteristics of a zeolite and simply quantifying instead how its topology affects the free energies of formation of the various reactants, intermediates and products involved (that is, the free-energy landscape of the reacting system), we can identify the fundamental interactions and processes that control the shape selectivity of a particular transformation.

We focus our discussions on hydroconversion reactions to make this point, but emphasize that the 'free-energy landscape approach' has some important limitations. Most importantly, it can be applied in a straightforward fashion only to simple reactions that occur at a single reaction site; if reaction pathways are more complex (an example being competing reactions occurring at single active sites or at pairs of such sites), then our simple approach may no longer apply. We also note that zeolites obviously catalyse many chemical reactions other than hydroconversion, and it is well known that different reaction classes are often efficiently catalysed by zeolites that differ in their basic chemical composition. For example, whereas hydroconversion requires aluminosilicates, shape selective oxidation reactions are in general catalysed by titanosilicates (zeolites in which the framework $Si^{4+}$ is replaced by $Ti^{4+}$ rather than $Al^{3+}$). The chemical characteristics of zeolites thus clearly play an important role in achieving the desired catalytic activity. But within each general class

## Box 2 | Simulating molecules in zeolites

The starting point of the simulation is the known crystal structure of the zeolite[37], from which we can generate the positions of the Si and O atoms. In our simulations we use periodic boundary conditions such that we mimic a perfect, infinitely large crystal without an external surface. As in most simulations studies, the zeolite is assumed to be rigid. Comparison of the results for flexible and rigid zeolites shows that flexibility has very little effect on the thermodynamic properties[38], but might be more significant for the transport properties because flexibility may increase or decrease the free energy barrier for diffusion[39]. Even for diffusion these effects are too small and experiments are not sufficiently accurate to assess the conditions under which the assumption of a rigid zeolite structure may break down.

### Configurational-bias Monte Carlo

Adsorption isotherms, which indicate the number of adsorbed molecules as a function of the pressure (chemical potential) of the gas in contact with the zeolite, are readily obtained from a Monte Carlo simulation in the grand-canonical ensemble in which temperature and chemical potential are imposed and the number of adsorbed molecules is a result of the simulation[40]. To change the number of adsorbed molecules, one of the Monte Carlo moves involves an attempt to add or remove a molecule. Such a move is subsequently accepted or rejected with a probability that depends on whether it has a favourable energy. Adding a molecule at a random position in the zeolite will only result in an acceptable conformation if it does not overlap with one of the atoms of the zeolite. For methane this is relatively easy and may only need the generation of, say, ten positions before an empty spot is found. Ethane requires two empty spots and so the number of attempts will be of the order of a hundred. Clearly, for the long-chain hydrocarbons of interest for catalytic applications the probability of generating a configuration in which none of the atoms overlaps is prohibitively small.

The CBMC technique has been developed[41,42] to make the insertion of these long-chain molecules possible. In a CBMC simulation a molecule is not inserted at random but grown atom by atom using a method based on an algorithm developed by Rosenbluth and Rosenbluth[43]. During the growing step, overlap with the zeolite atoms is avoided by selecting (from the set of possible positions at which to add the next atom) the position with the lowest energy with the highest probability of acceptance. The conventional Monte Carlo scheme, however, relies on randomly generated configuration and hence the bias in the growing scheme towards configurations with the lowest energy would result in a scheme that would generate configurations that do not have a proper Boltzmann distribution. The key aspect in a CBMC simulation is that we compute the bias introduced in the growing step and this information is used in an acceptance rule that exactly removes this bias[42]; that is, the product of the probabilities of growing a particular configuration and of acceptance recovers the correct Boltzmann distribution.

The CBMC can be used in various ensembles. Combined with the grand-canonical ensemble, it allows for the insertion and deletion of long-chain hydrocarbons and hence the computation of a complete adsorption isotherm. CBMC allows us also to compute free energies. For small molecules, free energies are conveniently computed using the Widom test particle insertion method[40]. In this method, the free energy is expressed as an ensemble average related to the Boltzmann factor of the energy $U^+$ of randomly inserted test particles that probe the energy, but do not participate in the simulation: $\langle \exp(-U^+/k_BT) \rangle$. For chain molecules, random insertion results in configurations that almost always overlap and hence have zero contribution to the ensemble average. Here, the CBMC scheme also allows the generation of biased configurations that do not overlap and hence have a non-zero contribution to this ensemble average. Also, for the free energy it is important to correct for the bias in the growing scheme[37].

### Intermolecular potentials

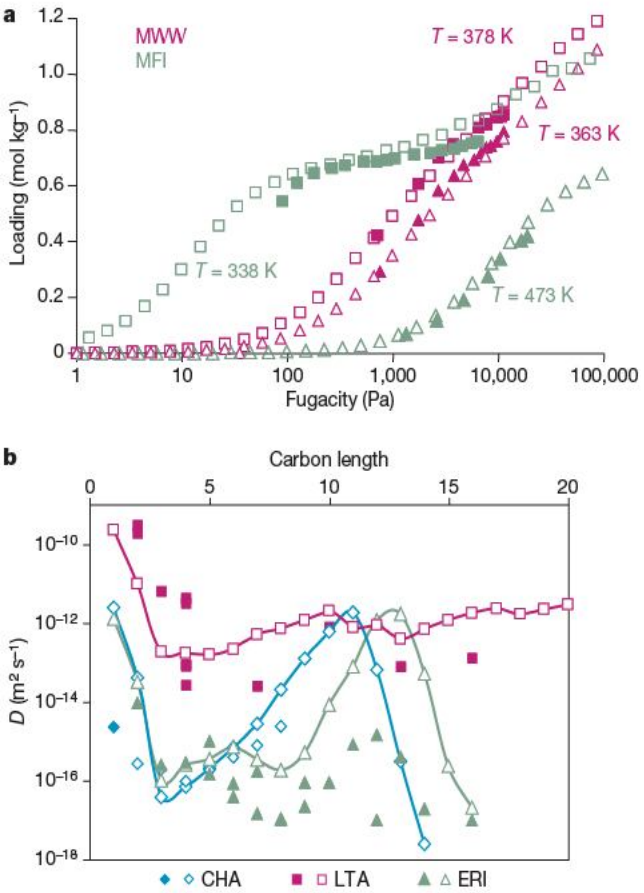
For adsorption of hydrocarbons in zeolites it is convenient to use a united-atom description, in which the $CH_i$ groups are seen as one united atom. The united atoms are connected with bond-bending, bond vibrations and torsion potentials such that an accurate description of the internal structure of a hydrocarbon is made. The non-bonded, van der Waals interactions are optimized to reproduce the vapour–liquid phase behaviour of the alkanes[44,45]. The interactions of the Si, O and Al atoms of the zeolite with the united atoms of the hydrocarbon are described with Lennard–Jones parameters[46]. These parameters have been optimized to accurately reproduce the steps that observed in the experimental adsorption isotherms[46].

### Adsorption isotherms and diffusion coefficients

Typical examples of the accuracy that can now be achieved for computing thermodynamic and transport data are shown in Box 2 Figure a and b, respectively. The intermolecular interactions have been optimized for different molecules and/or materials and therefore the results shown in these figures demonstrate that one can predict adsorption isotherms[46] and diffusion coefficients[14] of long-chain hydrocarbons in various zeolites. Molecular simulation studies have predicted many different phenomena, such as commensurate freezing[12] and entropic separation[47], that were only recently confirmed by experiments[48,49].

### Rare events simulations

A very small diffusion coefficient often is the result of molecules that are trapped in low (free) energy sites and only once in a while hop to another adsorption site by crossing a free-energy barrier that separates these two sites. In a rare event simulation this hopping rate is computed in two steps; first, the probability that a molecule can be found on top of the barrier, followed by a separate simulation in which the probability is computed so that this molecule actually reaches the other adsorption site[40]. The probability of finding a molecule on top of the barrier can be computed directly from the free-energy profile, which is the free energy as a function of the position of the molecule in the zeolite. The second step involves a large number of very short molecular dynamics simulations in which a molecule is initialized on top of this barrier and the probability is determined that this molecule does end up in the neighbouring adsorption site and does not return to its original location. As this involves a simulation that starts on top of the barrier, it is much faster than the time it takes a molecule to 'climb' this barrier. These rare event methods have been applied to zeolites at low[14] (see Box 2 Figure b) and even high loadings[13].




**Box 2 Figure | Thermodynamic and kinetic data from molecular simulations.** Typical examples of the application of molecular simulation; the adsorption isotherms (**a**) have been obtained via CBMC simulations and the diffusion coefficients (**b**) by rare events simulations. Comparison with the experimental data (filled symbols) illustrates the agreement that can be obtained. **a**, The adsorption isotherms give the loading of *n*-hexane in MWW and MFI as a function of the fugacity (pressure) at different temperatures (data from ref. 50). **b**, Diffusion coefficients *D* of the *n*-alkanes in the CHA, LTA and ERI as a function of chain length (data from ref. 14).

of reactions (for example, hydroconversion, or hydrocarbon oxidation), the free-energy landscape approach to understanding shape selectivity should hold; that is, among all zeolites with a chemical composition appropriate for a particular hydrocarbon conversion process of interest, this approach can be applied to identify those zeolites that have a topology optimally suited to generating the maximum yield of the desired product(s).

The zeolite-catalysed conversion of *n*-decane illustrates the free-energy landscape approach. This conversion involves many competing reactions (illustrated in Fig. 1), and a first step in identifying the preferred reaction path(s) in such a complex system—and hence the dominant product(s)—is the quantification of the system's free-energy landscape, and in particular how this landscape changes with zeolite topology. The challenge here is that although the gas-phase free energies of formation for most molecules in the reaction scheme of Fig. 1 are known and similar, the free energies of formation of the molecules when present in the adsorbed phase in a zeolite are rarely known. Exceptions are molecules that cannot react because they do not fit inside a zeolite pore; they exhibit a prohibitively large positive free energy of formation. In the case of zeolites with very wide pores, adsorbed molecules will be in physical equilibrium with the gas phase and will probably be unaffected by condensed-phase thermodynamic constraints. But when the fit becomes snugger, molecules formed inside the zeolite may no longer be able to desorb as products and products that have left the zeolite may no longer be able to re-adsorb; that is, molecules are locked in or locked out. Under such conditions, the gas phase and adsorbed phase can no longer equilibrate and the free-energy landscape imposed by the zeolite topology on the reacting system will leave its signature on the product distribution. Such lack of equilibration between gas phase and adsorbed phase is endemic to larger molecules in industrial processes such as we consider here. We note that even though complete equilibrium will never be achieved (as in almost all processes), almost all thermodynamic arguments intrinsically assume equilibration. In the context of the present discussion, we argue that despite the lack of full equilibration, a quantification of the adsorbed-phase free-energy landscape nevertheless serves as a useful starting point that can help us to arrive at a quantitative description of zeolite catalysis.

Quantification of the free-energy landscape associated with a particular zeolite topology and particular hydrocarbons has long been impossible, but can now be achieved using sophisticated simulation methods (see Box 2). We are accordingly able to compile data such as are shown in Fig. 2a, which illustrate how the free energy of formation of five intermediates involved in *n*-decane hydroconversion changes relative to that of *n*-decane as a function of zeolite structure. We note that the relative adsorption coefficients of the five intermediates adsorbed on the zeolite pore walls are a measure of the ease with which the individual intermediates form (relative to *n*-decane), and not of their proton affinity[4] or intrinsic reactivity. Compared with the corresponding gas-phase values, which are nearly identical for all isomers, FAU (a zeolite with large cages) has little effect on the relative free energy of formation of all five molecules investigated. By contrast, the zeolite TON (with narrow channels) makes a prohibitively high and positive contribution to the free energy of formation of di- and tri-branched *n*-decane isomers. This effect quantitatively confirms the traditional concept of shape selectivity, which predicts that di- and tri-branched isomers will not form because they are too large for the TON pores.

But a comparison of *n*-decane conversion in the zeolites MFI and MEL illustrates the limitations of the traditional shape selectivity concept and the importance of quantitative free-energy data: despite their similar structures, the zeolites generate markedly different product distributions[15]. Both zeolites can accommodate all the molecules involved in *n*-decane conversion that are shown in Fig. 1, and these may desorb after their formation as final products or serve as reaction intermediates and react further. The probability of a particular molecule forming during a zeolite-catalysed process is directly proportional to its free energy of formation in the adsorbed phase. In the case of *n*-decane conversion, this free energy is dominated by the zeolite contribution unless zeolites with very wide pores such as FAU are used (as discussed above).

The data in Fig. 2 illustrating this contribution for key reaction intermediates[16] clearly indicate that 4,4-dimethyl octane is the most stable species in MFI whereas 2,4-dimethyl octane is the most stable

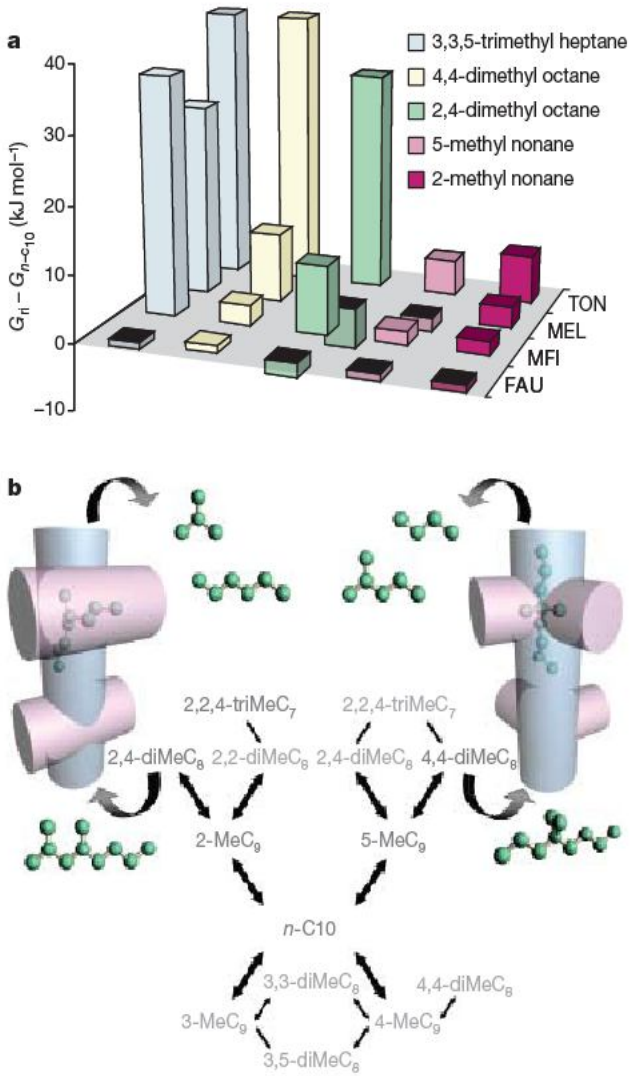



**Figure 2 | Schematic representation of the free-energy model.** The contribution of the zeolite topology to the free energy of formation of key reaction intermediates of the hydroconversion of *n*-decane in different zeolite structures (data taken from ref. 16). **a**, The free-energy differences between the reaction intermediates and *n*-decane for different types of zeolites: $G_{ri} - G_{n-C_{10}}$. TON is a small-pore zeolite that greatly increases the free-energy differences (for the tri- and di-branched isomers the free energies are 95.8 and 123.6 kJ mol$^{-1}$, respectively, off the scale of the figure). FAU is a large-pore zeolite that contributes little to the free-energy differences. MEL and MFI have similar structures to each other but with pore widths intermediate between those of TON and FAU. The contributions of MEL and MFI are similar for most reaction intermediates, but there are marked differences in free energies for some specific reaction intermediates. The consequences for the reactions in the pores are illustrated in **b**. MFI (the structure on the right) prefers to form 4,4-dimethyl octane because it is commensurate with the zig-zag channel and hence forms a nice fit (see the molecule in the zeolite), whereas MEL (the structure on the left) prefers to form 2,4-dimethyl octane, which snugly fits in the larger intersection. As a consequence, the reaction paths in MFI are dominated by the path *n*-$C_{10} \rightarrow$ 5-Me$C_9 \rightarrow$ 4,4-Me$C_8$, whereas in MEL the dominant path is: *n*-$C_{10} \rightarrow$ 5-Me$C_9 \rightarrow$ 2,4-Me$C_8$. The reaction scheme shown in Fig. 1 shows that 2,4-Me$C_8$ and 4,4-Me$C_8$ are cracking precursors that yield branched and linear butane, respectively. This explains why MEL produces more isobutane relative to butane and why MFI exhibits the opposite trend in the experimental product distribution[15].

species in MEL. The dominant reaction path involving di-branched isomers thus proceeds through two different intermediates in MFI and MEL (4,4-dimethyloctane and 2,4-dimethyloctane, respectively). Rare event simulations of these systems have shown[17] that the free-energy barriers for the hopping of both intermediates from one pore intersection to another are so high that the diffusion coefficients are impossibly small; in other words, neither molecule can leave after it has been produced inside the zeolite, so that equilibration with the gas-phase cannot be reached. Desorption of either hydrocarbon is only possible after cracking converts 4,4-dimethyl octane into *n*-butane product in the case of MEL, or 2,4-dimethyl octane into isobutane product in the case of MFI. This comparative example illustrates how zeolites can control product distributions by favouring the formation of particular reaction intermediates. We also note that both reaction intermediates are typical 'ship-in-a-bottle' molecules that can form inside a zeolite but not desorb. Any attempt to experimentally determine the adsorbed-phase free energies of such molecules is a major challenge. Simulations may thus be the only viable means for obtaining the information that is needed to develop a mechanistic explanation for the different catalytic properties of topologically similar zeolites such as MEL and MFI.

The zeolite-catalysed hydroconversion of *n*-hexadecane ($n$-$C_{16}$) provides another illustration of the insights to be gained from the use of simulations and quantitative free-energy considerations. Empirical evidence has taught us that to maximize the production of molecules with the highest octane number (that is, di-branched hexane ($C_6$) isomers) and to minimize the formation of low-octane hexane isomers (especially *n*-hexane), the conversion needs to be carried out in the presence of zeolite catalysts with one-dimensional, tube-like pores[18]. Interestingly, the free energies of formation of the molecules involved in the conversion suggest that at very low reactant concentrations, the selectivity of the process changes little with the pore width of the zeolites used[17]. This observation is at variance with experimental results. However, the actual process is carried out at high pressures where the zeolites are completely filled, and calculations carried out under these conditions predict product selectivities that agree with observed product distributions. In this case, the pressure-dependence of the simulation results signals that the selectivity of the process is an entropic effect: the high operating pressure favours formation of those $C_6$ isomers that can optimally pack inside the zeolite[19]. Subsequent desorption of $C_6$, followed by readsorption and further reaction could in principle obscure this effect. But the long $C_{16}$ reactant molecules block any re-adsorption within the filled zeolite pores and prevent equilibration with the gas phase, thereby ensuring that the relative abundance of the various $C_6$ products leaving the reactor bears the signature of the relative entropy of the $C_6$ isomers in the adsorbed phase. As a result, the selectivity of the *n*-hexadecane ($n$-$C_{16}$) hydroconversion process can be optimized by using zeolite structures with pore diameters that allow for the most efficient packing of branched $C_6$ molecules.

The above examples illustrate that quantitative thermodynamic and kinetic data are essential to move from intuitive yet speculative explanations for shape selectivity to a firm mechanistic understanding. In fact, several shape-selective transformations catalysed by zeolites have been re-analysed using the principles we have outlined above, resulting in the identification of selectivity mechanisms different from those proposed originally[19–23].

## *In silico* screening

As illustrated in the preceding section, computer simulations are now at a stage where they can accurately quantify the free-energy landscape imposed by a given zeolite topology on a reacting system and thus help us to develop mechanistic explanations for why a reaction of interest yields the product distribution that is experimentally observed. But a more challenging question is whether this simulation-based methodology has predictive power; that is, whether it can screen zeolite structures to identify those particularly well suited for new applications. A case in point is hydrodewaxing, an important refining process that converts the longest hydrocarbons present in a fuel or lubricant feed into smaller molecules and thus eliminates the risk of wax precipitation and associated engine problems during later use. In hydrodewaxing, the zeolite catalyst thus needs to convert the longest hydrocarbons while leaving shorter hydrocarbons unharmed. Expressed in terms of free energies of formation, the zeolite should have a topology that maximizes the difference between the free energies of formation of the molecule to be converted, say $n$-$C_{25}$, and a reference molecule that needs to remain untouched, such as $n$-$C_{10}$.

The result of such 'screening by computer' (summarized in Fig. 3) shows that the optimally performing zeolites are ZSM-48, MTW, GON, SFE and OFF. These zeolites all have pores with a typical tubular character, and pore diameters that are optimum for absorbing the long wax molecules that need to be converted during hydrodewaxing. The use of zeolites SFE and OFF in this context has been explored[24,25] but not pursued owing to practical difficulties with synthesis, whereas ZSM-48 and MTW are at the heart of intellectual property activity[26–28] on hydrodewaxing applications. Interestingly, GON had not yet been considered in this context before the computer screening, but a patent application has now been filed for a dewaxing process based on GON[29]. Similarly, computer screening also identified STI for dewaxing[52].That patent applications can be entirely based on molecular simulations illustrates the considerable progress that has been made in this field since Thomas and coworkers carried out the first simulation on hydrocarbon behaviour in a zeolite.

## Simplification to success

We believe that the explanation and prediction of shape selectivity on the basis of a quantitative free-energy landscape is a widely applicable and useful approach for understanding zeolite catalysis. But it is based on some important assumptions that should be kept in mind. As mentioned before, the simulations we have discussed all assume that once a particular family of zeolite with suitable chemical features has been identified as a promising type of catalyst for a given class of reactions, topology will control catalyst performance and the exact chemical composition of the zeolite will have only a minor influence. This allows us to compute and compare the free energies of

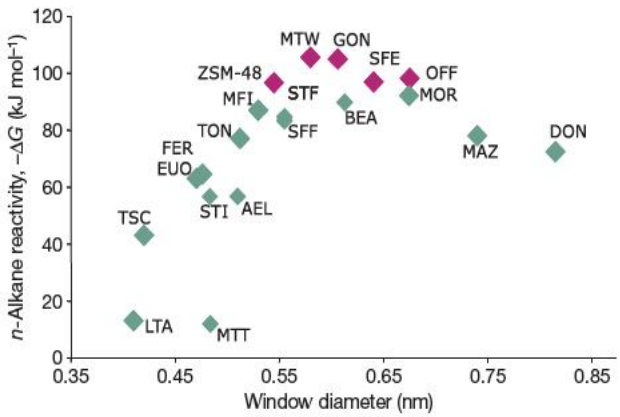



**Figure 3 | Zeolite screening by computer.** For hydrodewaxing, the optimal reactivity of a structure can be expressed in terms of the free energy of adsorption $\Delta G$ of a long *n*-alkane ($n$-$C_{25}$). Frequently, zeolites are characterized by their window diameter, that is, the smallest diameter molecules 'see' when moving through the material. Topologies that exhibit a highly negative value preferentially convert long instead of short *n*-alkanes, that is, they optimally remove the waxes and leave the shorter hydrocarbons. Indirect experimental evidence for the validity of this thermodynamic approach is the match between the optimum structure and the focus of a recent flurry in patenting activity. The topologies in red have been patented by Shell[26] and ExxonMobil[27] and GON has recently been 'discovered' by simulations[29].

formation of reactants, intermediates and products in various silica zeolite structures without explicitly considering the chemical reactions as such. The fact that our approach is successful despite such a striking—to many readers, perhaps even alarming—simplification can be explained in part by the findings of very accurate quantum chemical calculations[30,31]: detailed investigations of how zeolite-catalysed reactions proceed show that the nature of the acid site is remarkably similar for zeolites with markedly different topologies. That is, the acid sites in, say, narrow-pore or large-pore zeolites differ little in their reactivity.

The exact location of acid sites is known for a few zeolites and has been used when calculating the free energies of formation for a number of hydrocarbons in these zeolites[32]. The results support the assumption that the difference between the free energy of formation of different hydrocarbons in the same zeolite is almost unaffected by the exact location—and even the presence—of acid sites; that is, free-energy differences depend almost exclusively on zeolite topology. All computations of free energies of formation are based on free-energy differences between the molecule that is formed and a reference molecule, so simulations aimed at determining free-energy differences can use all-silica structures as excellent approximations of acid zeolites and thus avoid the complex problem[33] of having to account for the exact location—or at least the distribution—of acid sites.

Another important assumption underlying the free-energy landscape approach is that the Brønsted–Evans–Polanyi relationship applies. This relationship holds that the free-energy barrier that needs to be surmounted to form a given molecule from its starting material is proportional to the free energy of formation of that molecule. Put differently, the impact of a given zeolite topology on reaction kinetics mirrors its impact on the free energy of formation of the molecules that are formed. The Brønsted–Evans–Polanyi relationship is based on the simple idea that the transition state of a reaction largely resembles the product molecule, and is well known to be valid for a wide range of simple reactions such as hydroisomerization and hydrocracking that occur at single catalytic sites. But reaction pathways can be complex and, for example, require the involvement of more than one catalytic site. In such instances, the Brønsted–Evans–Polanyi relationship will often break down and the free-energy landscape approach to probing zeolite catalysis will no longer be applicable. In this context, we also note that if the Brønsted–Evans–Polanyi relationship does hold, the question of whether or not complete equilibrium is reached during reaction is not particularly important because the reaction kinetics is directly coupled to thermodynamics. This direct coupling means that quantitative information on the impact of the zeolite on the free energies of formation provide a reliable indication of which product molecules will form preferentially.

As the preceding discussion has clearly shown, the free-energy landscape approach to understanding—and even predicting—shape selectivity in zeolite catalysis is based on a number of drastic simplifications. But the success of the approach, as illustrated by the specific examples mentioned in this Review, emboldens us to conclude that a fairly simple but accurate and quantitative thermodynamic analysis will often suffice to explain in detail the product distribution characteristics of particular processes catalysed by a given zeolite. At present, the simulations that enable these thermodynamic analyses involve a significant idealization of industrial catalysis, for they do not consider catalysis occurring at the outside of zeolite crystals, nor zeolite crystal defects, stacking faults or intergrowths. But the fact that the simulated results compare well with experimental reference data obtained for nearly perfect zeolite crystals is encouraging. The next step will be systematically to include in simulations the effect of imperfections on the thermodynamic and transport properties, thus allowing us to describe not only ideal zeolite catalysts but also industrial zeolite-based catalysts. It should also be possible to expand the use of the free-energy landscape approach to zeolite-catalysed reactions other than hydroconversion reactions. Hydroconversions have been the focus of this Review for the simple yet practically important reason that a wealth of experimental data exists that documents shape selectivity for this reaction type; for most other reactions, shape selectivity has not been established unambiguously.

## To the future

The approximately 180 zeolite structures known to exist constitute only a very small fraction of the more than 2.5 million structures that are feasible on theoretical grounds[34]. Such a database of hypothetical zeolite structures has been regarded an important step towards "designer catalysts"[35], and it can in principle be screened for zeolites that are suitable for particular applications using the same methodology used to screen existing zeolites for their hydrodewaxing performance. To cope with such a large number of structures and to identify efficiently those with useful and superior catalytic properties will clearly involve enormous computational challenges. But even if effective screening is accomplished and successfully used to identify novel catalysts that allow us to use increasingly scarce fossil fuels more efficiently, any such *in silico* promise can only be realized if it is also possible to synthesize the identified structures. We expect that computer simulations will prove invaluable in this regard as well, by delivering increasingly detailed mechanistic insights into the nucleation and crystal growth of zeolites[36] that might eventually allow us to rationally control and guide these processes such that they form desired new zeolite structures.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ARTICLES

# The dynamics of measles in sub-Saharan Africa

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**Although vaccination has almost eliminated measles in parts of the world, the disease remains a major killer in some high birth rate countries of the Sahel. On the basis of measles dynamics for industrialized countries, high birth rate regions should experience regular annual epidemics. Here, however, we show that measles epidemics in Niger are highly episodic, particularly in the capital Niamey. Models demonstrate that this variability arises from powerful seasonality in transmission—generating high amplitude epidemics—within the chaotic domain of deterministic dynamics. In practice, this leads to frequent stochastic fadeouts, interspersed with irregular, large epidemics. A metapopulation model illustrates how increased vaccine coverage, but still below the local elimination threshold, could lead to increasingly variable major outbreaks in highly seasonally forced contexts. Such erratic dynamics emphasize the importance both of control strategies that address build-up of susceptible individuals and efforts to mitigate the impact of large outbreaks when they occur.**

The interruption of measles transmission in some parts of the industrialized world is a triumph of public health[1]. Global measles immunization programmes have focused on increasing routine vaccine coverage in young children through the World Health Organization (WHO) Expanded Programme on Immunization (EPI)[2]. EPI is reinforced by wide age range Supplementary Immunization Activities (SIAs) aimed at eliminating susceptible individuals who persist in the population beyond the age recommended for vaccination through routine health services[2,3]. Recent increases in vaccine distribution through the Measles Initiative, a partnership of WHO, UNICEF, the American Red Cross, the United Nations Foundation, and the US Centers for Disease Control, have led to an estimated 60% reduction in measles mortality worldwide relative to the global burden of mortality in 1999 (ref. 2). However, measles remains a leading cause of vaccine-preventable death in children under 5 yr in much of the world (particularly parts of sub-Saharan Africa and southeast Asia)[4]. The continued persistence of measles in these low income, high birth rate countries reflects the challenges of achieving high vaccine coverage in areas with limited public health infrastructure. Major epidemics still occur and Outbreak Response Vaccination (ORV) is one of the strategies that may be deployed to mitigate the immediate morbidity and mortality impact of these occasional outbreaks[5–12].

The epidemic dynamics of measles are the best understood among acute infections[2,13–21]. Powerful herd immunity leads to a tendency for multi-annual outbreaks, forced mainly by seasonal variations in infection rate (owing to schooling patterns in industrialized countries), and generating large, characteristically biennial, epidemics in the pre-vaccination era[13,16,22]. The resulting deep inter-epidemic troughs can cause local stochastic extinction of infection in towns below a critical community size (CCS) of 300–500 thousand in Europe and North America[23]. This emphasizes the epidemiological impact of spatial heterogeneity in host distribution, which can also drive complex spatiotemporal epidemic patterns[1,16]. Demographic heterogeneities in the recruitment of susceptible individuals (owing to birth rate variations, vaccination and the age structure of transmission) also strongly impact epidemic dynamics[21,24–26]. Finally, in theory, strong seasonal forcing can drive chaotic dynamics in the measles attractor[27]. However, in practice, measles dynamics and persistence in industrialized countries are more consistent with weaker seasonality, driving epidemic limit cycles, moulded by demographic heterogeneities in space and time[15,28,29].

Previous analyses of measles dynamics have shown how seasonality in transmission and birth rates can interact to generate complex multi-annual outbreak dynamics[15,28]. The impact of demographic variations is perhaps best shown in the dynamic transition from annual to biennial cycles of measles outbreaks in England and Wales[30], driven by the decrease in birth rates following the post-World War II baby boom. In countries where birth rates are much higher, the standard SIR model parameterized on observations from industrialized countries predicts highly persistent, annual dynamics in large towns[21,31]. However, the following analysis of measles time series in Niger and its capital city, Niamey, reveals starkly contrasting patterns to such extrapolations.

Niger presents an important opportunity to understand the dynamics and control of vaccine-preventable childhood infections in a high birth rate country—a critical issue, given that this is the typical host demography in countries where these infections remain major public health problems. Niger is in the western Sahel and ranges from several densely populated cities in the south to desert climates in the north, sparsely populated by nomadic pastoralists. The country's population is approximately 13 million and its birth rate is among the highest reported in the world, at 50.73 births per year per 1,000 population[32]. Routine single-dose measles vaccine distribution through EPI was initiated in 1987. Niger's first measles-only SIA, targeting all children aged 9 months to 14 yr, was conducted in 2004 and achieved an estimated coverage of 99% of the target population[3]. Before the SIA, measles outbreaks exhibited

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annual cycles at the national scale (Fig. 1a inset), as expected, with regular timing and somewhat variable amplitude (Fig. 1a). This large-scale pattern is consistent with dynamics in the region (for example, Burkina Faso[33] or Cameroon[34]). However, our analysis of the temporal dynamics and spatial synchrony of measles outbreaks at the local scale reveals that the appearance of regular, annual outbreaks is an artefact of averaging erratic and asynchronous local epidemics (Supplementary Information C). There is one regularity, however: the timing of measles outbreaks invariably coincides with the end of the annual rainy season (Fig. 1a), which is the dominant seasonal driver in the region.

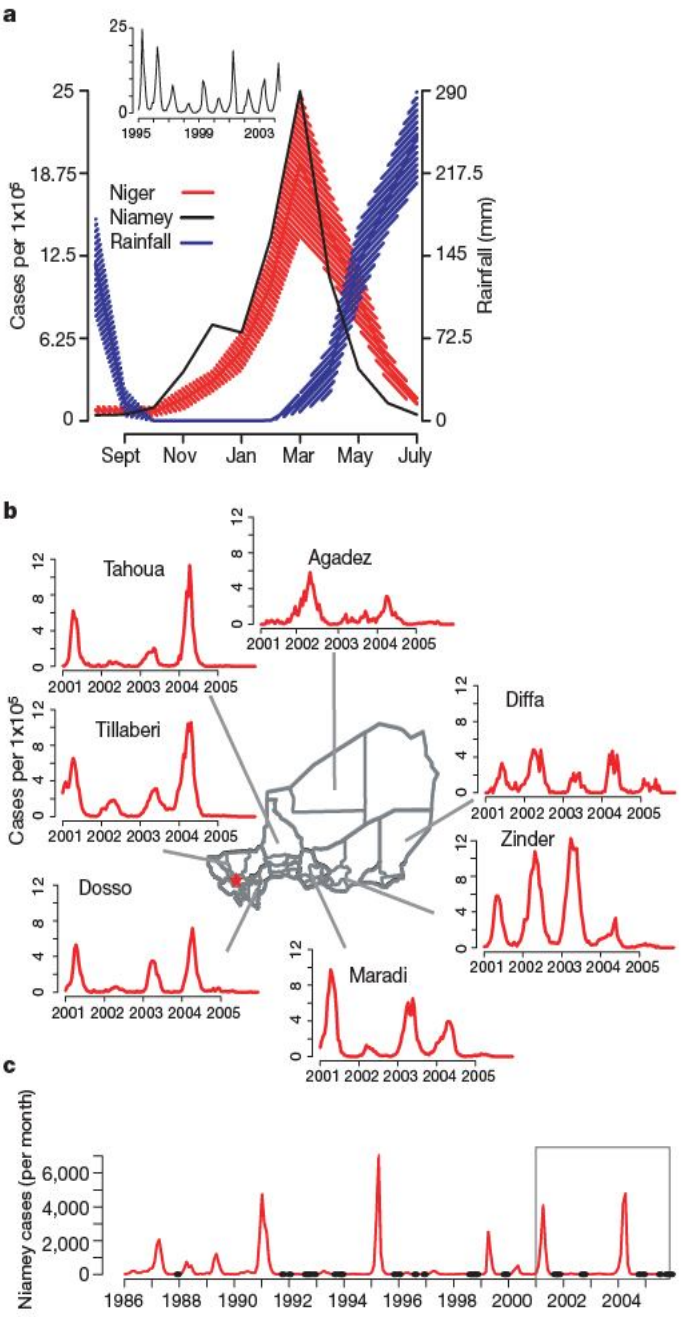


**Figure 1 | Time series dynamics of measles outbreaks in Niger.** **a**, Mean number of reported measles cases per 10,000 nationwide in Niger from 1995 to 2004, and the mean monthly rainfall over the same time period (blue). Shaded regions give ±2 standard deviations. Black curve, mean monthly cases of measles in Niamey from 1986 to 2005. Inset, monthly measles time series from 1995 to 2004. **b**, Weekly measles case reports from seven departements of Niger, 2001–2005. Red asterisk, Niamey. Each departement is an aggregate of 3–8 arrondissements. **c**, Case reports per month for the city of Niamey from 1986 to 2005. The box indicates the time frame shown in **b**. Black dots, months with 0 reported cases.

## Seasonality and dynamics in Niamey

For model parameterization, we focus on the relatively well-documented time series of incidence from 1986 to 2002 (before the national SIA) from Niger's capital city, Niamey (Fig. 1c)—a city of approximately 750,000 persons (according to the 2001 National Census), which is twice the historical CCS for measles in Europe and North America[23,35]. On the basis of lessons from Europe and North America and given Niger's high birth rate, we would expect persistent annual measles cycles[21,31]. In contrast, empirical patterns over the last 30 yr testify to highly erratic outbreaks; monthly case reports from 1986 to 2004 reveal occasional large outbreaks followed by years of very few cases (Fig. 1c). Similarly, annual measles incidence rates in Niamey between 1975 and 1985 ranged from 1–5%[36], consistent with this irregular pattern.

Measles epidemics in Niamey decline at the onset of the rainy season, regardless of the magnitude of the outbreak (Fig. 1a, c). This indicates that powerful seasonal forcing of transmission may be driving irregular, fragile dynamics even in such a large, high birth rate population. We explore this issue using a stochastic time series Susceptible–Infected–Removed (TSIR) epidemiological modelling framework, which has been applied successfully to measles dynamics elsewhere[22,25,30]. The TSIR model allows us to estimate the form of seasonality in transmission (below). First, however, we use sinusoidal forcing[15] to illustrate the general dynamical consequences of varying seasonal amplitude. Figure 2a shows a bifurcation diagram for a simple deterministic TSIR model with a fixed, 14-day infectious period and sinusoidal forcing in transmission rate[15], as a function of seasonal amplitude and birth rate. At low seasonal amplitude (Fig. 2a, seasonality = 0.2), the dynamics resemble historical patterns in the industrialized world (for example, in London): a dynamic transition from annual to biennial cycles as birth rate declines from high levels[15]. In contrast, at high seasonal amplitude (Fig. 2a, seasonality = 0.6), corresponding to that which we estimate for Niamey (Fig. 2b; see below), the range of birth rates at which the system exhibits stable 1–4 yr cycles decreases and the dynamics become chaotic over a broad range of birth rates. Further, as birth rate and strength of seasonality increase, the depth of the inter-epidemic trough becomes very shallow (to the right of the dashed contour in Fig. 2a), greatly increasing the likelihood of local stochastic extinction.

We estimate seasonal variation in the transmission rate in Niamey explicitly by applying the TSIR model to 17 yr of monthly data from the city (Fig. 1c). To account for uncertainty in the reporting rate, we use a bayesian state space approach (Methods). The estimated seasonality in the transmission rate shows a single peak, roughly in antiphase to the seasonal rainfall profile (Fig. 2b). A possible mechanistic explanation for this pattern is the increase in urban density in the dry season owing to seasonal migration from outlying agricultural areas[37]. Niamey's pattern of measles seasonality is conspicuously different from the school-term forcing observed before mass-vaccination in England and Wales that is due to mixing of children in schools (Fig. 2b)[30]. This difference is also associated with contrasting age–incidence profiles: the median age of measles infection in Niamey is less than 2 yr[36,38], compared to 4–5 yr for the England and Wales epidemics[39].

The magnitude of transmission seasonality in Niamey is fourfold that of historical London (Fig. 2b). This puts the Niamey dynamics in a large amplitude biennial regime (Fig. 2c), well within the predominantly chaotic region of parameter space for a broad range of birth rates (Fig. 2a). The strong seasonality leads to deep inter-epidemic troughs (Fig. 1c), making long-term local persistence of measles in Niamey very unlikely. The model predicts that, even for very large populations (>5 million), long-term persistence is unlikely without external reintroduction (Supplementary Information A). Thus, the CCS for measles persistence in Niamey is over an order of magnitude higher than predicted from classical studies[23,35]. There is also significant regional heterogeneity in this stochastic fragility; relative to their size, Niamey and the communities in the neighbouring regions of

Dosso and Tillaberi exhibit many more fadeouts (weeks with zero cases reported) than departements such as Maradi and Zinder (Fig. 3a).

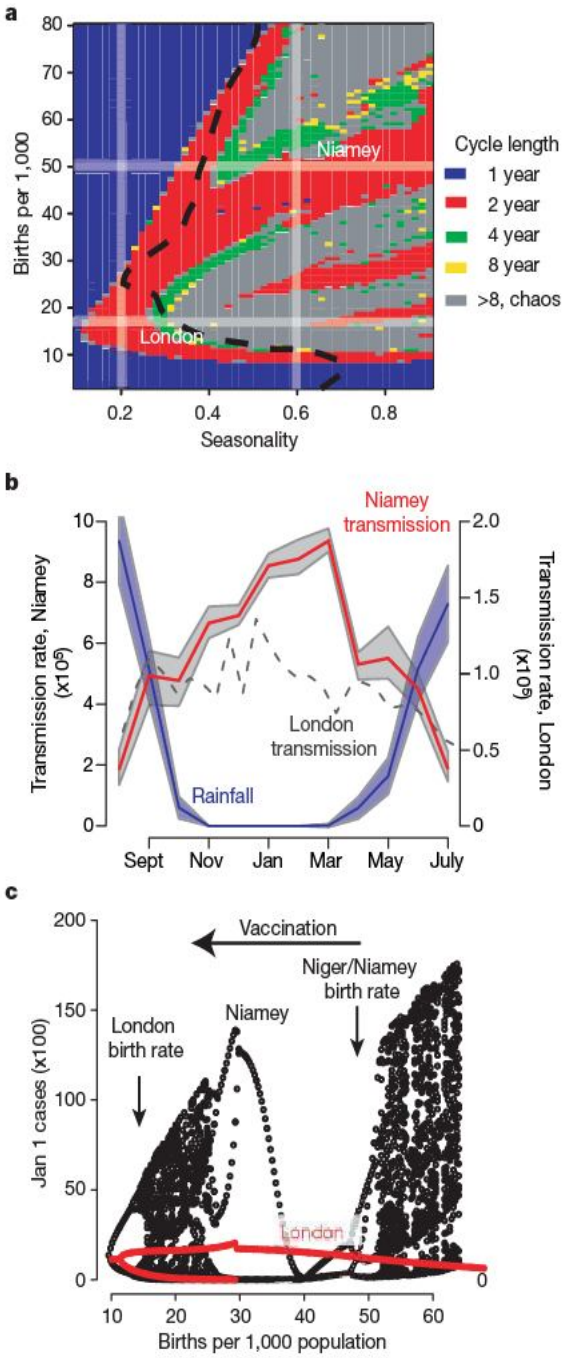


**Figure 2 | Dynamic consequences of seasonal variation in measles transmission rate in Niger.** **a**, Bifurcation diagram for a deterministic seasonally forced TSIR epidemic model. Seasonal transmission is modelled as a cosine wave; $\beta(t) = (\text{mean } \beta)(1+\alpha\cos(2\pi t))$. $x$ axis, the amplitude of the seasonal forcing, $\alpha$; $y$ axis, annual birth rate per 1,000 people in the population. Colours indicate the periodicity of the epidemic dynamics. Black dashed contour, the range of parameter space above which the minimum number of cases is <1; that is, persistence is unlikely in a stochastic setting. Vertical lines, approximate seasonal amplitude of pre-vaccine London and Niamey (assuming sinusoidal forcing); horizontal lines, the approximate birth rates for both countries. **b**, Estimated seasonal transmission rate for Niamey (solid red line). Shaded grey regions, the 95% bayesian credible intervals; blue line, the mean annual rainfall per month, with ±2 standard deviations indicated with blue shading; dashed line, the seasonality (scaled for population size) for pre-vaccine London for comparison. **c**, Bifurcation diagram for the estimated seasonal transmission rate for Niamey (black) as a function of birth rate per 1,000. The bifurcation pattern for pre-vaccination London (red) is given for comparison[15]. Increased vaccination coverage has the consequence of decreasing the effective birth rate and may lead to increasingly erratic dynamics (horizontal arrow).

## Measles metapopulation dynamics

These patterns suggest the following picture of national (metapopulation) measles dynamics in Niger. Strong seasonality leads to frequent local extinction of measles at the onset of the rainy season. The relatively low connectivity in the regional metapopulation (Supplementary Information C) results in infrequent local reintroductions; this episodic coupling leads to inter-epidemic periods of unpredictable length and frequency, during which the population of susceptible individuals can grow sufficiently to fuel large magnitude outbreaks. We explore this picture using a stochastic multi-patch version of the TSIR model. The 39 arrondissements and Niamey are represented as patches, connected by stochastic dispersal with a kernel that is a power function of distance among patches parameterized to the observed correlation from 2001 to 2005 (Methods). We assume the same seasonal pattern of transmission in all patches, scaled to maintain a constant $R_0$. The model supports our dynamical hypothesis, capturing the qualitative pattern of episodic outbreaks at the local scale (Fig. 1b, c), and seemingly annual dynamics at the aggregate regional scale (Fig. 1a, inset). Furthermore, although it was parameterized on the basis of observations from 1986 to 2002 in Niamey, the metapopulation model predicts the qualitative pattern of regional persistence at the national scale from 2001 to 2005 (Fig. 3). In particular, the model accurately predicts relatively low persistence in the remote north and relatively higher persistence in the central regions of Maradi and Zinder (see Supplementary Information C for further discussion of the model and parameter fitting). Overall, the combination of strong seasonal forcing and weakly connected metapopulation patches generates a setting in which outbreaks of variable frequency and magnitude are the rule rather than the exception.

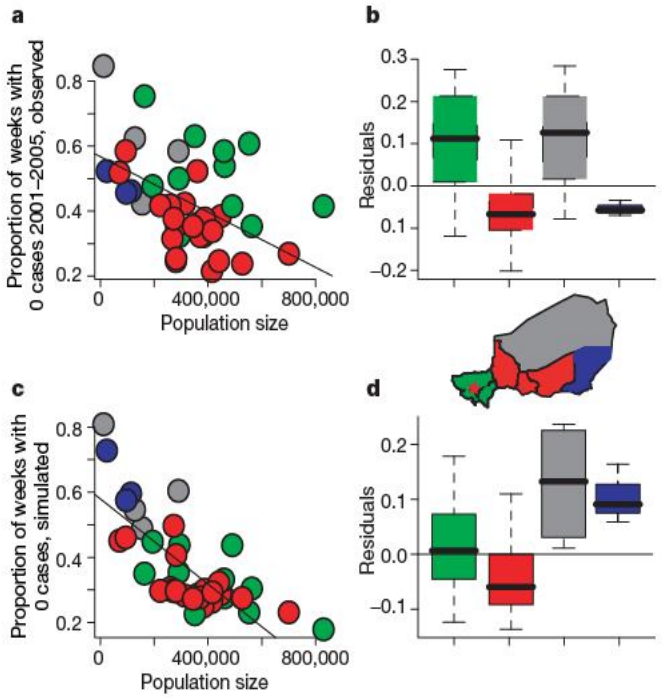


**Figure 3 | Observed and predicted patterns of measles persistence in Niger.** **a**, Proportion of weeks with 0 cases reported in 39 arrondissements plus Niamey plotted as a function of population size. Solid line, the mean relationship (excluding Niamey as an outlier). Arrondisements are colour coded by region. Green dots, the departements of Tillaberi and Dosso surrounding Niamey; red dots, the departements of Tahoua, Maradi and Zinder; grey and blue dots, the sparsely populated departements of Agadez and Diffa respectively (map inset). **b**, Boxplots of residuals from **a**, grouped by region (colours match map inset). Box, the interquartile range; whiskers, the range. Heavy line, the median. **c**, Proportion of weeks with 0 cases reported in 39 arrondissements plus Niamey plotted as a function of population size, as simulated from the metapopulation model. **d**, Boxplots of residuals from **c** (colours match map inset, boxes as in panel **b**).

### Implications for measles control

The erratic outbreak dynamics in highly seasonal, high birth rate settings present a challenge for measles control. In particular, the strong seasonality suggests that deterministic epidemic dynamics will remain in the large amplitude (possibly chaotic) regime even as overall vaccine coverage increases (Fig. 2c). Major outbreaks can quickly overwhelm local public health resources and result in high rates of childhood mortality[3]. Thus, to mitigate the potentially devastating effects of these outbreaks, both surveillance to detect epidemics, and the right balance between routine, supplementary and reactive control strategies are key to long-term measles control strategies in the region. We now use our metapopulation model to explore these issues.

### Outbreak detection in Niamey

Even for very erratic epidemics, the strong transmission seasonality in Niamey can help in predicting the annual start of outbreaks. Active monitoring of cases early in the high transmission season (September–November) strongly predicts historic outbreak size (Supplementary Information B). In an interesting parallel study[40], it was recently shown that the timing of the prior year's epidemic peak may be diagnostic of the level of susceptibility and help to predict future outbreak size in certain seasonal and chaotic systems. This relationship is only weakly predictive in our system, because high birth rates and exceedingly strong and sharply focused seasonality lead to highly synchronized epidemic peaks over a range of outbreak magnitudes (Supplementary Information B). As such, there is little power in the timing of peaks to predict the magnitude of subsequent outbreaks in this region.

Since 2004, the vaccination strategy in Niger has changed to incorporate periodic (3–4 yr interval) SIAs. Although this programme is too recent for us to evaluate the effect on seasonality and predictability on the basis of incidence data, simulation results suggest timing of the onset of outbreaks will not change, even under an established SIA programme (Supplementary Information E).

### Optimal vaccination

We focus initially on the balance between routine immunization and ORV (reflecting the situation in Niger up to 2004). We initially assume that routine immunization was applied to the entire metapopulation at the relevant rate; in contrast, ORV, targeting all children regardless of immune status (that is, 6 months to 14 yr, following the recommendations of ref. 8), was applied on 15 November only to the large Niamey-like patch in response to an outbreak, defined as 10 total cases in October (Supplementary Information B). Given the costs (human, logistic and financial) of mounting such a campaign we assume conservatively that ORV campaigns would not be conducted in consecutive years. We restrict our discussion to the outbreak dynamics in the large, Niamey-like patch.

Simulations stress the intuitive result that increased routine vaccination coverage reduces the mean number of cases per year (Fig. 4a). Importantly, simulations also predict that increased routine vaccination and ORV will alter the dynamics of major outbreaks (>2,500 cases), by reducing the rate at which measles will be reintroduced to Niamey following local extinction. Specifically, this reduction in the regional flux of infection results in longer intervals between major epidemics (Fig. 4c) and therefore larger epidemics, when they occur (Fig. 4d). On the face of it, Fig. 4 also implies that high levels of ORV can interact with lower levels of background vaccination (40–70%) to generate a plateau (Fig. 4a, *) or increase (Fig. 4b, *) in average cases and in large, rare epidemics (Fig. 4d,

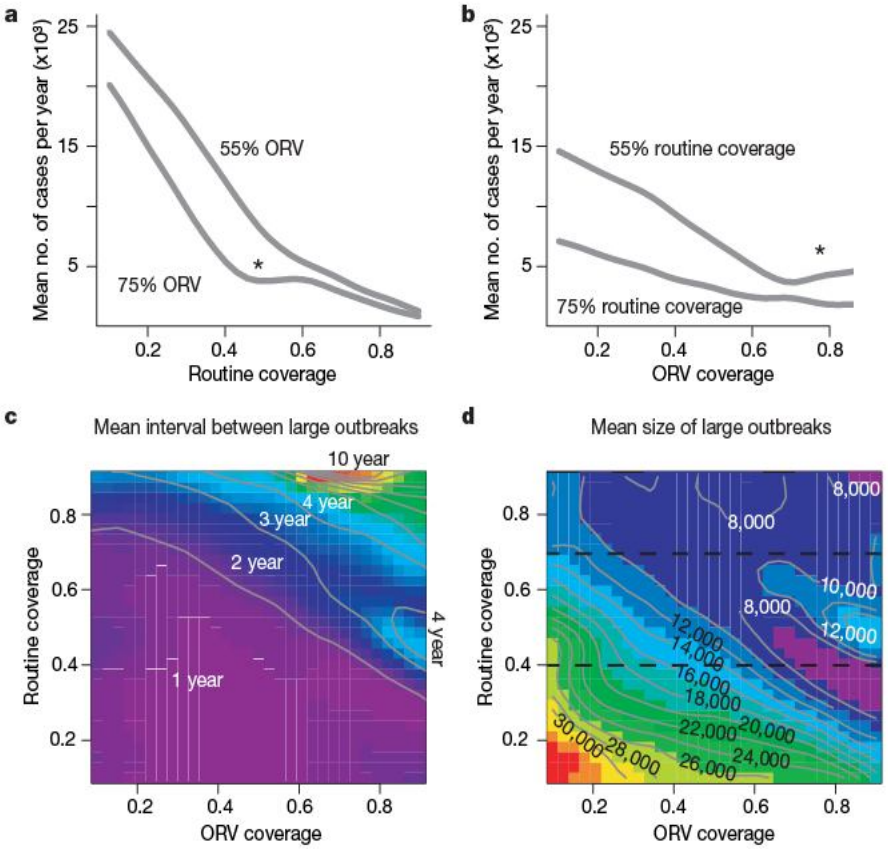


**Figure 4 | Impact of vaccination programmes on outbreak magnitude and frequency in the large patch for the metapopulation model.** **a**, Mean annual measles cases as a function of routine vaccine coverage for low (55%) and high (75%) levels of ORV coverage. The mean is taken over 50 yr of a stochastic simulation. **b**, Mean annual measles cases as a function of ORV coverage for low (55%) and high (75%) levels of routine vaccine coverage. The mean is taken over 50 yr of a stochastic simulation. **c**, Mean interval between large outbreaks (frequency) as a function of routine and ORV coverage in a Niamey-like patch. The mean is taken over 50 yr of a stochastic simulation using the estimated seasonal transmission rate for Niamey. **d**, Mean size of large (>2,500 cases) outbreaks as a function of routine and ORV coverage in a Niamey-like patch. The mean is taken over 50 yr of a stochastic simulation using the estimated seasonal transmission rate for Niamey.

between the dashed lines). However, this simply reflects the fact that, although ORV can partially compensate for low herd immunity at low routine immunization, occasional large epidemics would 'escape' this control. Overall, increasing levels of routine vaccination and ORV reduce average incidence. We predict lower incidence to interact with strong seasonal forcing in this weakly coupled metapopulation to generate large, unpredictable epidemics even at vaccination levels just below the regional eradication threshold (Supplementary Information C, D).

Given the recent introduction of SIAs in Niger, it is important to establish how periodic vaccine pulses affect the above picture. Because there has only been one SIA in Niger (in 2004), it is difficult to calibrate a detailed model of future supplementary immunization in the country. Preliminary simulations of pulsed supplementary immunization for Niger (Supplementary Information E) indicate that they can be effective, in reducing both the average number of cases and also the probability of very large, unpredictable epidemics. The major effect here is achieved by increasing average coverage and imposing a multi-annual forcing that generates more predictably spaced outbreaks (Supplementary Information E).

## Discussion

The high seasonality of transmission in Niamey leads to more irregular measles dynamics than predictions that are based on historical data for industrialized countries in the northern hemisphere. This emphasizes the potential dangers of extrapolating dynamics for these sorts of highly non-linear systems without a detailed understanding of local parameters. Interestingly, although poliovirus in India exhibits similarly strong seasonality, its longer infectious period leads to more regular annual dynamics than measles[41].

The quality of the Niger data allows us a rare opportunity to generate data-driven models for measles metapopulation dynamics in the region. This analysis reveals highly non-linear behaviour, in the chaotic region of epidemic periodicity, revisiting a previous debate in population dynamics[29, 42]. The resulting high amplitude outbreaks interact with demographic stochasticity and low metapopulation coupling to generate fragile dynamics; this is reflected in a CCS for measles persistence in Niamey over an order of magnitude higher than the standard figure for less seasonally forced settings.

The key to measles eradication is to bring vaccine coverage up to the level of herd immunity[2,24,43]. This goal may be achieved through a routine two-dose vaccine schedule, as is the case in much of the industrialized world[2]; our results also stress the importance of ORVs for responding to large outbreaks in this highly seasonal setting. However, the complex, high-amplitude dynamics that result from a combination of strong seasonality and high birth rates lead to erratic boom and bust outbreaks that are likely to continue even as routine vaccination coverage improves. Increasing routine vaccination is dynamically equivalent to a reduction in birth rate[15] and may thus be expected to move the Niamey dynamics, at least initially, more firmly into the chaotic regime (Fig. 2a, c).

The optimal strategy for administering a second dose as a function of the local epidemiological environment is an important area for future research. Preliminary results indicate that regular, pulsed vaccine programmes, like SIAs, may lead to more regular dynamics (Supplementary Information E, see also refs 44 and 45), but are unlikely to eliminate major outbreaks until baseline vaccine levels reach high levels. Thus, surveillance and reactive campaigns may also be of increasing importance to mitigate the morbidity and mortality impact of large irregular outbreaks as routine vaccine coverage approaches the WHO goals for 2010 (ref. 2). The use of regional coordination of SIAs to minimize buildup of susceptibility, and the potential for re-introduction of the measles virus following local eradication, is an important consideration as regional immunization strategies are developed. Simple rules, such as thresholds for outbreak detection (Supplementary Information B) and strategies for susceptible minimization are key to optimizing intervention strategies. To this end, dynamic models rooted in local data are important tools for providing clear recommendations for control strategies.

## METHODS SUMMARY

**Estimating seasonality.** We estimated the seasonal variation in transmission rate by fitting a TSIR model with imperfect binomial reporting to the 17-yr-long time series of monthly incidence in Niamey (1986–2002) using bayesian Markov chain Monte Carlo methods[46]. These data are before regional SIAs (the first in December 2004) or local ORVs in Niamey (2004; ref. 8). The unobserved time series of measles cases was specified as a TSIR model: $I_{t+1} \sim \mathrm{NB}(\beta_m S_t I_t^{\alpha}, I_t)$ where $\mathrm{NB}(a,b)$ signifies a negative binomial process[13]. $S$ and $I$ indicate the number of susceptible and infected hosts, respectively, $\beta_m$ indicates the month-specific transmission rate and $\alpha$ is a tuning parameter to account for non-linearities in transmission. The time step was taken as 0.5 months, so that the TSIR model can be coupled to a binomial observation model in which the observed number of cases each month is distributed as binomial$(I_{t-1}+I_t, P_{obs})$, where $P_{obs}$ is the reporting probability for cases. Additional information is given in Supplementary Information F.

**Metapopulation model.** We evaluated the effect of vaccination on outbreak dynamics using a metapopulation model consisting of 40 local communities representing the 39 arrondissements plus Niamey. We modelled coupling among patches as a power function of distance, parameterized on the basis of the 2001–2005 spatially resolved data[47] (Supplementary Information C). The strength and shape of seasonal forcing for all communities matched that estimated for Niamey, and scaled such that $R_0$ was constant. The birth rates were taken as that reported for Niger: 50.73 births per 1,000 individuals per year. Routine vaccination was assumed to target young children across the entire metapopulation. ORV vaccination campaigns targeting Niamey only were initiated if the number of observed cases in October exceeded 10 (assuming 50% reporting; Supplementary Information F) and the time since the last ORV campaign was at least 1 yr. ORV campaigns targeted all children of 6 months to 14 yr[8], and the vaccination target was assumed to be reached within two weeks[8].

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**Supplementary Information** is linked to the online version of the paper at www.nature.com/nature.

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LETTERS

# Slow dust in Enceladus' plume from condensation and wall collisions in tiger stripe fractures

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**One of the spectacular discoveries of the Cassini spacecraft was the plume of water vapour and icy particles (dust) originating near the south pole of Saturn's moon Enceladus[1–5]. The data imply considerably smaller velocities for the grains[2,5,6] than for the vapour[4,7], which has been difficult to understand. The gas and dust are too dilute in the plume to interact, so the difference must arise below the surface. Here we report a model for grain condensation and growth in channels of variable width. We show that repeated wall collisions of grains, with re-acceleration by the gas, induce an effective friction, offering a natural explanation for the reduced grain velocity. We derive particle speed and size distributions that reproduce the observed and inferred properties of the dust plume. The gas seems to form near the triple point of water; gas densities corresponding to sublimation from ice at temperatures less than 260 K are generally too low to support the measured particle fluxes[2]. This in turn suggests liquid water below Enceladus' south pole.**

The structure of Saturn's E ring clearly points at Enceladus as its main source[8,9] and the early prediction of cryo-volcanic activity[10–12] was recently confirmed by Cassini data[1–5]. Enceladus' plume originates from sources in the south polar region, located on four linear structures[5,13–15] (dubbed 'tiger stripes'), which probably form the outlets of a system of cracks in the moon's ice shell through which water vapour escapes to vacuum from subsurface sites of evaporation. Although the gas expands to space[4,7] with a speed of 300–500 m s$^{-1}$, most grains are ejected at speeds[2,5,6] smaller than

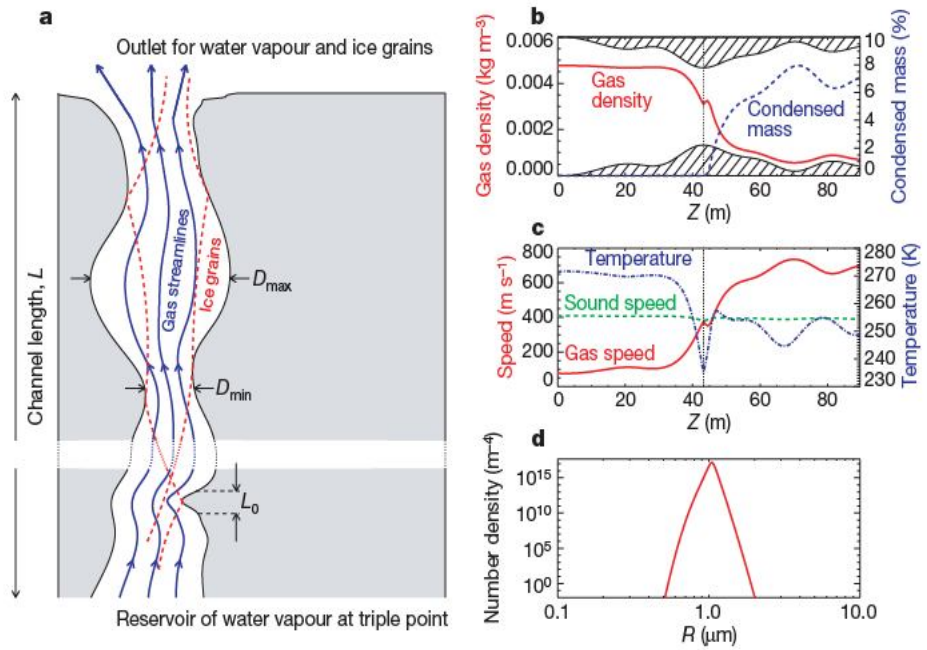


**Figure 1 | Gas flow and condensation in cracks in Enceladus' ice shell.** **a**, Schematic sketch. Gas escapes through channels of variable cross-section to vacuum from a reservoir at the triple point of water. Channel profiles are constructed as superposition of random harmonics (Supplementary equations S11–S14), with smallest length scale $L_0$, for a given total length $L$, and minimal and maximal channel widths $D_{min}$ and $D_{max}$. Because the equations describing the channel flow and condensation are invariant under the transformation $D \to \alpha D$, for any positive factor $\alpha$, we may choose $D_{max}$ arbitrarily for fixed $D_{min}/D_{max}$. Eventually, we identify $D_{max}$ with the collision length $L_{coll}$, which must be of the same order. From the condensation model the particle speed–size distribution is obtained as an average over an ensemble of 5,000 individual channel solutions. **b**, Typical solution for gas density and mass fraction of condensed grains along a channel of length 90 m, $D_{min}/D_{max} = 0.56$, and $L_0 = 18.76$ m. The position in the channel is denoted by $Z$. The density drops drastically near the narrowest point of the channel (nozzle throat), where most grains nucleate. **c**, Profiles of gas speed and temperature. The transition to supersonic flow occurs near the nozzle throat, displaced slightly downstream owing to condensation. Cooling in the nozzle zone leads to a drastic increase of supersaturation and condensation. Owing to the latent heat, the temperature rises again. **d**, Particle number density per radius increment for one single channel. Other random channels (varying $L_0$ and $D_{min}/D_{max}$) yield distributions with peak sizes between tens of nanometres and tens of micrometres (Supplementary Fig. 3).

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Enceladus' escape velocity of 240 m s$^{-1}$. Such a difference would appear plausible for a non-stationary (for example, explosive) process but it is not expected for the observed quasi-stationary particle[15] and gas fluxes. Moreover, neither this dynamical difference nor the dust formation may be attributed to processes outside the satellite: here the gas is extremely dilute and practically collision-free[4,7]. Hence the grains must form inside the satellite.

Existing models suggest that simultaneous freezing and boiling of near-surface water[5,6], suddenly exposed to vacuum, leads to ejection of vapour and grains. However, in this framework the stationarity of the plumes is difficult to understand. Models based on decomposition of clathrates[16,17] can explain the observed abundances of volatile gases[3] but the formation of dust grains (speed and size distribution) is not quantified. Here we show that grain condensation in the subsurface gas flow is consistent with the observed properties of the dust plume[2,5], and the inferred gas speeds and production rates[3,4,7]. Just as in a nozzle, the variation of the channel width causes transition to supersonic speeds and locally enhanced condensation (Fig. 1). Moreover, in a non-straight channel the streamlines of dilute vapour and grains generally differ (Fig. 1a). Thus, directional changes of the gas flow (imposed by the cracks) and wall collisions will lead to an effective deceleration of grains relative to the gas.

We model this effect as a Poisson random process with flight times $t$ exponentially distributed as $\exp[-t/(L_{\mathrm{coll}}/u_{\mathrm{gas}})]$, where $L_{\mathrm{coll}}$ defines the characteristic length between collisions and $u_{\mathrm{gas}}$ is the gas speed. Grains are re-accelerated with a rate depending on gas density and particle radius $R$ (Fig. 2). We obtain (Supplementary Information) their velocity distribution $P(u_{\mathrm{grain}})$:

$$P(u_{\mathrm{grain}}) = \frac{R}{R_{\mathrm{c}}}\left[1+\frac{R}{R_{\mathrm{c}}}\right]\frac{u_{\mathrm{grain}}}{u_{\mathrm{gas}}}\left[1-\frac{u_{\mathrm{grain}}}{u_{\mathrm{gas}}}\right]^{\frac{R}{R_{\mathrm{c}}}-1} \quad (1)$$

and their average speed as a function of radius $R$:

$$\langle u_{\mathrm{grain}}(R)\rangle = \left(1+\frac{R}{2R_{\mathrm{c}}}\right)^{-1} u_{\mathrm{gas}} \quad (2)$$

where we define the critical radius $R_{\mathrm{c}}$:

$$R_{\mathrm{c}} \equiv \frac{\rho_{\mathrm{gas}}}{\rho_{\mathrm{grain}}}\sqrt{\frac{8k_{\mathrm{B}}T_{\mathrm{gas}}}{\pi m_0}}\left[1+\frac{\pi}{8}(1-\beta)\right]\frac{L_{\mathrm{coll}}}{u_{\mathrm{gas}}} \quad (3)$$

separating slow and fast particles (see Fig. 2 for notation). The condensation coefficient $\beta$, ranging[18,19] from 0.1 to 1, quantifies the adsorption of water molecules by growing grains, so that their growth rate is proportional to $\beta$. For gas near the triple point of water and $L_{\mathrm{coll}}$ of the order of decimetres we find $R_{\mathrm{c}}$ in the submicrometre range. In this case submicrometre-sized dust particles escape the vent essentially at the gas speed, while larger grains are slower, with $\langle u_{\mathrm{grain}}(R)\rangle \propto 1/R$.

The expanding vapour is super-saturated and by condensation its density will adjust rapidly close to the saturated value $\rho_{\mathrm{eq}}(T_{\mathrm{gas}})$ at temperature $T_{\mathrm{gas}}$ (Fig. 2). Using equations (2) and (3) we obtain a relation between the collision length $L_{\mathrm{coll}}$ and temperature, which shows that $\langle u_{\mathrm{grain}}\rangle$ increases with $L_{\mathrm{coll}}$. Basically, $\langle u_{\mathrm{grain}}\rangle$ is determined by the brightness gradient of the plume[6]. To fix ideas, we take $\langle u_{\mathrm{grain}}\rangle = 100$ m s$^{-1}$ for micrometre-sized grains, which, as shown below, is consistent with plume brightness and observed particle number density (Fig. 3). In this way we obtain a lower bound for possible $L_{\mathrm{coll}}$ (Fig. 2). If the collision length, roughly determined by the width of the cracks, is smaller than this bound, the gas is too dilute to support the observed particle flux. For gas temperatures below ~170 K the mean free path must exceed 10 km, which requires implausibly wide or straight cracks. More plausible channel widths between decimetres and metres, implying collision lengths of the same order, require temperatures between 240 and 260 K (Fig. 2). The expanding gas cools by a few tens of degrees (Supplementary Fig. 3), so that the actual temperatures at the site of evaporation must be 260 K or higher. Hence, a crack width of decimetres (Fig. 3) implies the presence of liquid water. We note that this conclusion is independent of the process of grain formation.

The large surface temperatures near the 'tiger stripes'[13,14] and evidence for hot chemistry[20] provide additional support for the presence of liquid water in equilibrium with ice and vapour (the triple point) below the south polar terrain[5,21,22]. Hence, we combine hydrodynamic equations and the first thermodynamic law to model condensation in water vapour (Supplementary Information) that expands from triple point conditions through channels of variable width (Fig. 1a). As the expanding gas cools, it becomes supersaturated, and efficient condensation into the solid phase sets in, typically limited to a narrow region (Supplementary Fig. 2). For the precise description of the homogeneous nucleation rate we employ a fit to experimental data for water[23] (Fig. 2). We obtain a closed set of equations (Supplementary equations S1–S4, S6, S7 and S10) for density, temperature and speed of the gas, as well as for the mass fraction and size of condensed grains along the channel (Fig. 1).

To account for natural geometric variety, we generate a large number (5,000) of random channels (Fig. 1). We combine the size distribution from individual channel profiles with the speed distribution (equation (1)) and average it over the ensemble of random channels. The resulting speed–size distribution is independent of the total channel length, provided the length is much larger than the maximal width of the cracks (Supplementary Fig. 7). From this distribution we generate starting conditions for three-body integrations and construct a computer model of the plume. Starting positions and directions are, respectively, uniformly distributed in a circular area of 10° half-angle around Enceladus' south pole and in a cone of 25° half-angle about the surface normal. Trajectories are followed numerically until the particles reach a distance of two Hill radii (sphere of gravitational influence, $r_{\mathrm{H}} = 948$ km) or they strike the moon again. The total flux of grains is normalized such that the model reproduces the peak number density 0.08 m$^{-3}$ of particles with $R > 1.6$ μm recorded by the Cassini High Rate Detector (HRD) on the trajectory of the

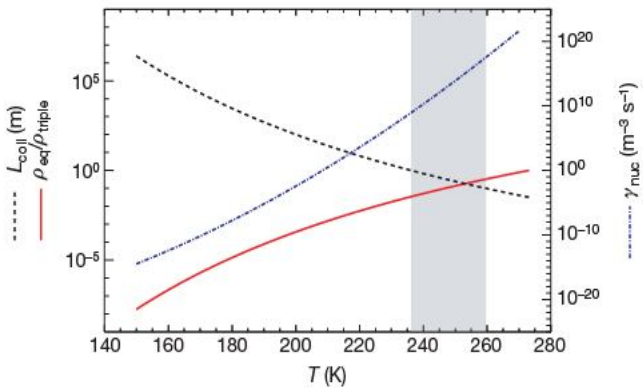


**Figure 2 | Temperature dependence of grain dynamics and homogeneous nucleation rate.** Ice grains move between random wall collisions according to the equation: $\dot{u}_{\mathrm{grain}} = \left(1+\frac{\pi}{8}[1-\beta]\right)\frac{\rho_{\mathrm{gas}}}{\rho_{\mathrm{grain}}}\sqrt{\frac{8k_{\mathrm{B}}T_{\mathrm{gas}}}{\pi m_0}}\left(u_{\mathrm{gas}}-u_{\mathrm{grain}}\right)$. For exponentially distributed flight times this yields the distribution (equation (1)) (Supplementary equations S17–S19). Here $\rho_{\mathrm{grain}} = 920$ kg m$^{-3}$ and $u_{\mathrm{grain}}$ are the material density and the speed of the grains, $\rho_{\mathrm{gas}}$, $u_{\mathrm{gas}}$ and $T_{\mathrm{gas}}$ are the density, the speed and the temperature of the gas, $k_{\mathrm{B}}$ is Boltzmann's constant, and $m_0$ is the mass of a water molecule. The dashed curve gives a lower bound for $L_{\mathrm{coll}}$ necessary to maintain a mean speed $\langle u_{\mathrm{grain}}\rangle$ for micrometre-sized particles that is larger than 100 m s$^{-1}$. We use $u_{\mathrm{gas}} = 500$ m s$^{-1}$ and $\rho_{\mathrm{gas}} = \rho_{\mathrm{eq}}(T_{\mathrm{gas}})$ (shown as solid red curve, $\rho_{\mathrm{triple}} = 4.85$ g m$^{-3}$), the saturated vapour density for given temperature. The temperature range for which $0.1\ \mathrm{m} < L_{\mathrm{coll}} < 1$ m is shaded in grey. For the homogeneous nucleation rate we use the relation $\gamma_{\mathrm{nuc}} = B(T_{\mathrm{gas}})(\sigma-1)^{n(T_{\mathrm{gas}})}$ with the supersaturation $\sigma = \rho_{\mathrm{gas}}/\rho_{\mathrm{eq}}(T_{\mathrm{gas}})$. The temperature dependence of the coefficients $B(T_{\mathrm{gas}})$ and $n(T_{\mathrm{gas}})$ (Supplementary equations S8 and S9) we obtain from fits to experimental data[23]. The nucleation rate drops drastically with decreasing temperature (dash-dotted curve, shown for fixed $\sigma = 10$).

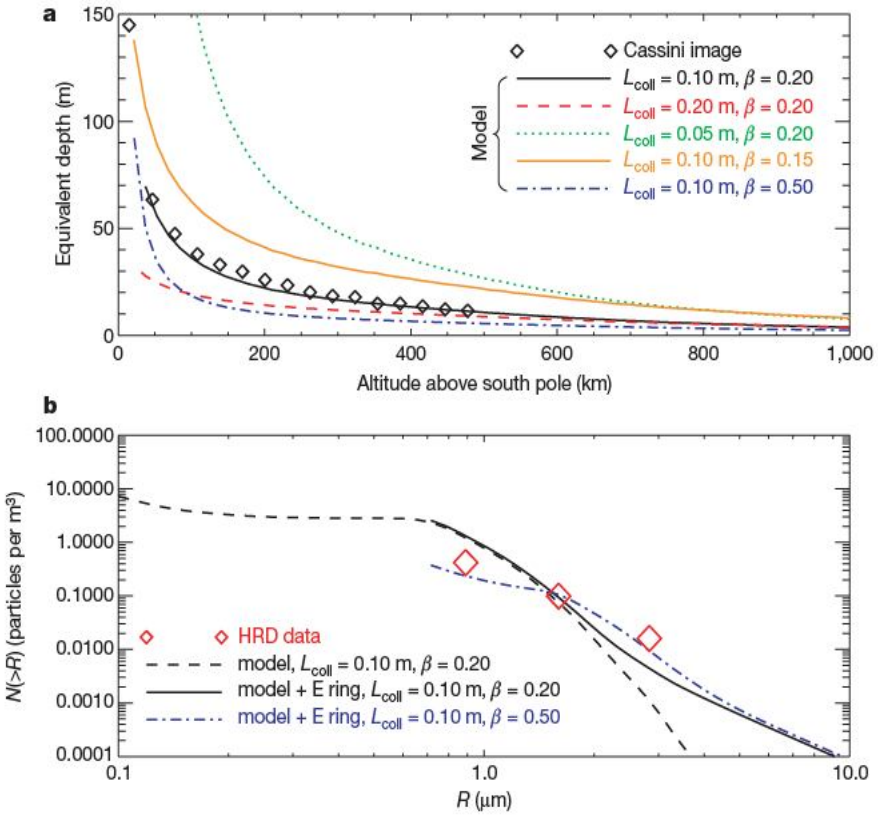


**Figure 3 | Comparison of model results with Cassini data.** **a**, Brightness of the model plume (phase angle 153°, wavelength 750 nm) with a Cassini image from February 2005 (N1487334245_1, phase angle 153°, IR1 filter $\lambda_{eff}$ = 751 nm). At this time Enceladus was near its pericentre, as it was at the time of the fly-by when the HRD data was recorded. Thus, the plume's level of activity should be comparable, if it varies with the orbital phase of Enceladus[27]. Background brightness due to E ring dust has been subtracted (Supplementary Information). Equivalent depth—that is, brightness integrated along image lines, $\int(I/F)dx$, orthogonal to the plume axis—is plotted versus altitude (symbols). The profile of equivalent depth for the model plume, computed from Mie theory, is overplotted (lines). The brightness of the model depends mainly on collision length $L_{coll}$ and condensation coefficient $\beta$ (see also Supplementary Figs 5 and 7). **b**, Cumulative particle size distribution from the model (dashed line) at the location where the HRD measured the maximal particle density (Cassini fly-by E11). The solid line includes an estimate for the background due to E ring particles[2,28] in the range $R > 0.8$ μm. The number densities $N$ of particles larger than 0.9, 1.6 and 2.8 μm, respectively, derived from the *in situ* data[2], are shown as diamond symbols.

Enceladus fly-by[2] in July 2005 (Fig. 3b). We reconstruct an image of the model plume (Supplementary Fig. 6) from Mie theory for smooth spheres (we use Mishchenko's Mie code: http://www.giss.nasa.gov/~crmim). The parameters $\beta$ and $L_{coll}$ are fixed by comparison to the brightness and the brightness gradient in Cassini images (Fig. 3a, Supplementary Fig. 6). A good agreement is obtained for $\beta = 0.2$ and $L_{coll} = 0.1$ m.

For the example shown in Fig. 3, the minimal widths and shortest scales (Fig. 1a) for the ensemble of random channels are drawn uniformly from the intervals $0.3 < D_{min}/D_{max} < 0.9$ and $5\,m < L_0 < 40\,m$ and the total length is fixed to $L = 150$ m. Variation of interval boundaries and channel length $L$ results in mild quantitative changes (Supplementary Fig. 7) of the curves of Fig. 3. A similarly small effect results from plausible variation of the area of particle ejection around the south pole and the width of the ejection cone.

With the HRD data we adjust the total dust flux, proportional to the venting-active surface, to obtain a value for this area of 200 m$^2$. A channel width ($D_{max} \approx L_{coll}$) of the order of 10 cm then yields a total venting active length of 2 km along the 'tiger stripes' (total length 500 km), consistent with the observation of isolated sources[13,15]. The total dust production rate is about 5 kg s$^{-1}$ of which ~10% escapes the satellite, in agreement with estimates of 1 kg s$^{-1}$ for the mass loss rate of the E ring[24]. Slightly more than half of the escaping mass is in particles larger than 1 μm. The mean fraction of condensed mass $\langle f \rangle \approx 0.06$ (Supplementary Fig. 3) then gives roughly 100 kg s$^{-1}$ of gas production, which is in reasonable agreement with the 150–300 kg s$^{-1}$ inferred from occultation data obtained by the Cassini Ultraviolet Imaging Spectrograph[4] and the Ion and Neutral Mass Spectrometer[3]. If this amount of gas condenses at the walls[22] of kilometre-long channels, we obtain a timescale of months for self-sealing. Our theory predicts particle sizes in the micrometre range (Fig. 3b, Supplementary Figs 4 and 5) with a local peak around 0.8 μm and a steep decay towards larger sizes. The HRD data shows a shallower slope due to the background of E ring particles: Large grains have larger orbital lifetimes[24–26] than small ones, and thus, once having escaped from the moon, they become more abundant in the E ring than in the plume.

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**Supplementary Information** is linked to the online version of the paper at www.nature.com/nature.

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LETTERS

# Phase diagram of a two-component Fermi gas with resonant interactions

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**The pairing of fermions lies at the heart of superconductivity and superfluidity. The stability of these pairs determines the robustness of the superfluid state, and the quest for superconductors with high critical temperature equates to a search for systems with strong pairing mechanisms. Ultracold atomic Fermi gases present a highly controllable model system for studying strongly interacting fermions[1]. Tunable interactions (through Feshbach collisional resonances) and the control of population or mass imbalance among the spin components provide unique opportunities to investigate the stability of pairing[2–4]—and possibly to search for exotic forms of superfluidity[5,6]. A major controversy has surrounded the stability of superfluidity against an imbalance between the two spin components when the fermions interact resonantly (that is, at unitarity). Here we present the phase diagram of a spin-polarized Fermi gas of $^6$Li atoms at unitarity, experimentally mapping out the superfluid phases versus temperature and density imbalance. Using tomographic techniques, we reveal spatial discontinuities in the spin polarization; this is the signature of a first-order superfluid-to-normal phase transition, and disappears at a tricritical point where the nature of the phase transition changes from first-order to second-order. At zero temperature, there is a quantum phase transition from a fully paired superfluid to a partially polarized normal gas. These observations and the implementation of an *in situ* ideal gas thermometer provide quantitative tests of theoretical calculations on the stability of resonant superfluidity.**

When the two spin components resonantly interact, the behaviour of the system becomes independent of the nature of the interactions. This case of unitarity has become a benchmark for experimental and theoretical studies over the last few years. However, there is an ongoing debate about the stability of resonant superfluidity, reflected in major discrepancies in predicted transition temperatures for the balanced spin mixture[7–9], and an even more dramatic discrepancy for the critical imbalance of the two spin components, called the Chandrasekhar–Clogston limit of superfluidity[2,3]. Recent quantum Monte Carlo calculations predicted that superfluidity would be quenched by a density imbalance around 40% (ref. 10), whereas other studies predicted a critical imbalance above 90% (refs 11–16). Our earlier work[17–19] suggested the lower limit but other experiments[20,21] were interpreted to be consistent with the absence of the Chandrasekhar–Clogston limit. This huge discrepancy reveals that even qualitative aspects, such as the role of interactions in the normal phase, are still controversial. The lack of reliable thermometry for strongly interacting systems limits the full interpretations of experimental results.

Here we resolve this long-standing debate by presenting the phase diagram of a spin-polarized Fermi gas at unitarity. We observe that the normal-to-superfluid phase transition changes its nature. At low temperature, the phase transition occurs with a jump in the spin polarization as the imbalance increases, which we interpret as a first-order phase transition. The local spin polarization or local density imbalance is defined as $\sigma = (n_\uparrow - n_\downarrow)/(n_\uparrow + n_\downarrow)$, where $\uparrow$ and $\downarrow$ refer to the two spin components with densities $n_{\uparrow,\downarrow}$. At high temperature, the phase transition is smooth and therefore of second order. The two regimes are connected by a tricritical point[4,22] and we estimate its position to be $(\sigma_{tc}, T_{tc}/T_{F\uparrow}) \approx (0.2, 0.07)$, where $k_B T_{F\uparrow} = \hbar^2(6\pi^2 n_\uparrow)^{2/3}/2m$ is the Fermi energy of the majority component of density $n_\uparrow$ ($k_B$ is the Boltzmann constant, $\hbar$ is the Planck constant divided by $2\pi$ and $m$ is the atomic mass of $^6$Li). Our low-temperature results confirm a zero-temperature quantum phase transition at a critical polarization $\sigma_{c0} \approx 36\%$.

This work required the introduction of several techniques. A tomographic reconstruction of local Fermi temperatures and spin polarization allowed us to obtain the phase diagram for the homogeneous system, no longer affected by the inhomogeneous density of the trapped samples. Furthermore, absolute temperatures were obtained using *in situ* thermometry applied to the non-interacting fully polarized Fermi gas in the outer part of the trapped samples, an ideal thermometer with exactly known thermal properties. Unlike previous work[18,23], this is a direct measurement without any approximations.

Our experiments are carried out in a trapping potential $V(\mathbf{r})$. The local chemical potential of each spin component is given as $\mu_{\uparrow,\downarrow}(\mathbf{r}) = \mu_{\uparrow 0,\downarrow 0} - V(\mathbf{r})$, where $\mu_{\uparrow 0,\downarrow 0}$ are the global chemical potentials. When $\mu_{\uparrow 0} \neq \mu_{\downarrow 0}$, owing to imbalanced populations, the chemical potential ratio $\eta(\mathbf{r}) = \mu_\downarrow/\mu_\uparrow$ varies spatially over the trapped sample and so, under the local density approximation, the trapped inhomogeneous sample is represented by a line in the phase diagrams of the homogeneous system. Figure 1 illustrates the spatial structure of a strongly interacting Fermi mixture in a harmonic trap. In the inner region, where $\eta$ is closer to unity, a superfluid with zero (or small) spin polarization will form at zero (or low) temperatures, having a sharp phase boundary against the partially polarized normal gas in the outer region. The spin polarization shows a discontinuity at the boundary of the superfluid core at $r = R_c$, a signature of the phase separation of a superfluid and a normal gas[24]. The critical polarization $\sigma_c = \lim_{r \to R_c^+} \sigma(r)$ represents the minimum spin polarization for a stable normal gas; $\sigma_s = \lim_{r \to R_c^-} \sigma(r)$ represents the maximum spin polarization for a stable superfluid gas. At higher temperatures, the discontinuity in the density imbalance disappears. The main result of this paper is the observation and quantitative analysis of such density profiles. Because we have no experimental evidence, we are not discussing the exotic partially polarized phases[25] which could exist only in the transition layer between the superfluid core and the normal outer region.

We prepared a variable spin mixture of the two lowest hyperfine states of $^6$Li atoms, labelled $|\uparrow\rangle$ and $|\downarrow\rangle$, at a magnetic field of 833 G. A

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broad Feshbach resonance at 834 G enhances the interactions between the two spin states. Our sample was confined in a three-dimensional harmonic trap with cylindrical symmetry. The *in situ* density distributions of the majority (spin ↑) and minority (spin ↓) components were determined using a phase-contrast imaging technique[19] (Fig. 2). We obtained the low-noise profiles $\tilde{n}$ by averaging the column density distribution along the equipotential line and determined the three-dimensional density profiles $n(r)$ using the inverse Abel transformation of the column densities $\tilde{n}(r)$ (see Methods Summary). Most of our measurements were performed at a total population imbalance of $\delta \approx 50\%$, where $\delta = (N_\uparrow - N_\downarrow)/(N_\uparrow + N_\downarrow)$ refers to the total numbers of atoms in the sample, $N_\uparrow$ and $N_\downarrow$ of the spin ↑ and ↓ components, respectively.

Figure 3 displays the radial profiles of the densities $n_{\uparrow,\downarrow}(r)$ and the corresponding spin polarization $\sigma(r)$ for various temperatures. The discontinuity in the spin polarization, clearly shown at very low temperatures, demonstrates the phase separation of the inner superfluid of low polarization and the outer normal gas of high polarization. At low temperature, the core radius $R_c$ is determined as the kink (and/or peak) position in the column density difference profile. At high temperature (but still in the superfluid regime), the discontinuity in $\sigma(r)$ disappears. At our lowest temperature, the radii of the minority cloud and the core region were measured as $R_\downarrow = 0.73(1)R_\uparrow$ and $R_c = 0.430(3)R_\uparrow$ (at $\delta = 44(4)\%$), respectively, and these values agree with recent theoretical calculations[10,25] within the experimental uncertainties due to the determination of $\delta$. Here, $R_\uparrow$ is the radius of the majority cloud, and the uncertainty of the final digit is indicated by parentheses.

We determined temperature from the *in situ* majority wing profiles. The outer part of the majority component, forming a non-interacting Fermi gas, fulfils the definition of an ideal thermometer, namely a substance with exactly understood properties in contact with the target sample. This new *in situ* method avoids the modification of the ideal gas profile caused by the collision with the inner core during ballistic expansion (ref. 18, see Supplementary Information). The outer part of the averaged column density difference profile ($r > R_\downarrow$) was fitted to a finite temperature Fermi–Dirac distribution in a harmonic trap (Fig. 4) and the relative temperature $T' \equiv T/T_{F0}$ was determined, where $k_B T_{F0} = \hbar^2(6\pi^2 n_0)^{2/3}/2m$ is the Fermi energy of the non-interacting Fermi gas, which has the same density distribution in the outer region as the majority cloud ($n_0$ is the central density of the non-interacting Fermi gas at zero temperature). We verified that anharmonicity of the trapping potential does not affect the fitted temperature (see Methods).

The critical lines of the phase diagram of a homogeneous spin-polarized Fermi gas were obtained by determining the local temperature and spin polarization at the phase boundary. The local relative temperature $T'_{local} \equiv T/T_{F\uparrow}$ was derived from the local density $n_\uparrow(R_c)$ according to $T'(R_c) = T/T_{F0} \times (n_0/n_\uparrow(R_c))^{2/3}$. Because we observe no jump in the majority density within our resolution, $T_{F\uparrow}$ is well-defined at the boundary. The critical polarizations $\sigma_c$ and $\sigma_s$ were measured as $\sigma_c = \sigma(R_c)$ and $\sigma_s = \sigma(R_c - 0.05R_\uparrow)$ (this criterion for $\sigma_s$ was more robust than a fitting procedure, but excludes the possibility that $\sigma_s$ will be equal to $\sigma_c$ at high temperature. Therefore, the measured $\sigma_s$ should be regarded as a lower bound for the polarization of

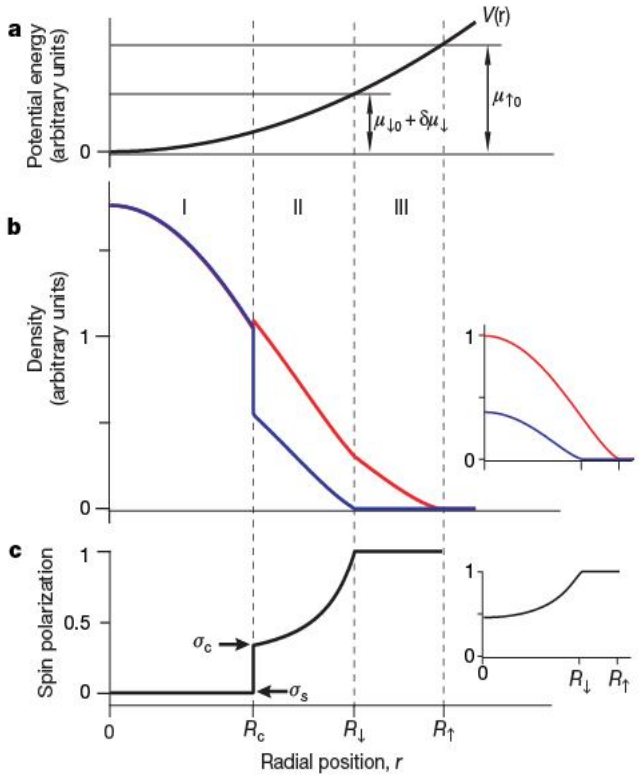


**Figure 1 | Schematic of spatial structure of a strongly interacting Fermi gas in a harmonic trap.** **a**, A two-component (spin ↑ and ↓) Fermi mixture is confined in an external potential $V(r) \propto r^2$ with the chemical potential $\mu_{\uparrow 0,\downarrow 0}$ of each spin component ($\delta\mu_\downarrow$ is the shift for the spin ↓ component owing to interactions). **b**, Density distributions of the majority component $n_\uparrow(r)$ (red line) and the minority component $n_\downarrow(r)$ (blue line). **c**, Spin polarization $\sigma(r) = (n_\uparrow - n_\downarrow)/(n_\uparrow + n_\downarrow)$. At zero temperature, the sample has a three-layer radial structure: (I), the core region ($0 \le r < R_c$) of a fully paired superfluid with $n_\uparrow = n_\downarrow$; (II), the intermediate region ($R_c < r < R_\downarrow$) of a partially polarized normal gas; and (III), the outer region ($R_\downarrow < r < R_\uparrow$) of a fully polarized normal gas. The critical polarization $\sigma_c$ (or $\sigma_s$) is defined as the minimum (or maximum) spin polarization of the normal (or superfluid) region. The non-interacting case is shown in the insets. The insets have the same axes as the main figure.

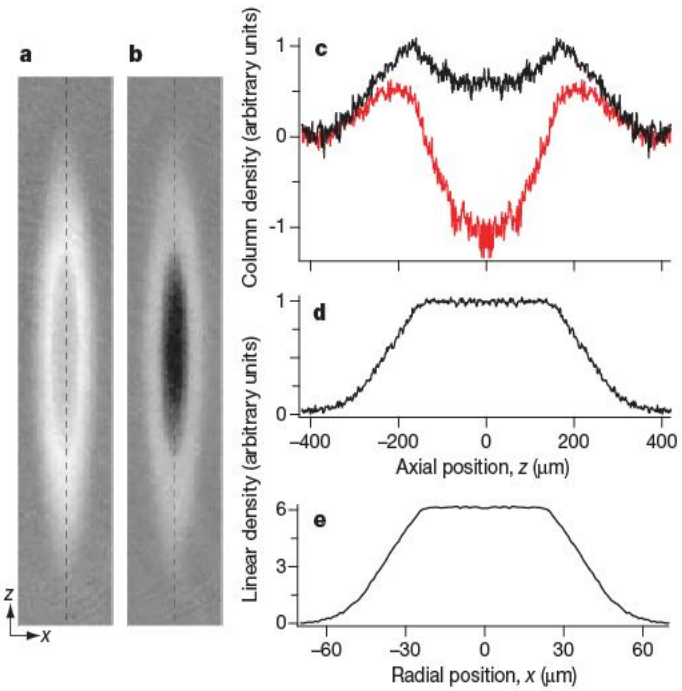


**Figure 2 | Double *in situ* phase-contrast imaging of a trapped Fermi mixture.** Two phase-contrast images of one sample were taken using different probe frequencies of the imaging beam, measuring the density difference $n_{d1} = n_\uparrow - n_\downarrow$ (**a**) and the weighted density difference $n_{d2} = 0.76\,n_\uparrow - 1.43 n_\downarrow$ (**b**), respectively. The images show the two-dimensional distribution of the column density difference, $\tilde{n}_{d1,2}(x,z) \equiv \int n_{d1,2}(\mathbf{r})dy$, owing to the line-of-sight integration. The field of view for each image is 150 μm × 820 μm. **c**, The distributions of the column density difference $\tilde{n}_{d1}$ (black line) and $\tilde{n}_{d2}$ (red line) along the central line (the dashed lines in **a** and **b**). The profiles of the integrated linear density difference, $\bar{n}_{d1,z} \equiv \int \tilde{n}_{d1}(x,z)dx$ (**d**) and $\bar{n}_{d1,x} \equiv \int \tilde{n}_{d1}(x,z)dz$ (**e**), show the identical flat-top feature except scaling. The aspect ratio of the trapping potential was $\lambda = 6.15$, the majority atom number was $N_\uparrow = 5.9(5) \times 10^6$, the population imbalance was $\delta = 44(4)\%$, and the relative temperature was $T' = T/T_{F0} = 0.03(1)$ (see text for definitions).

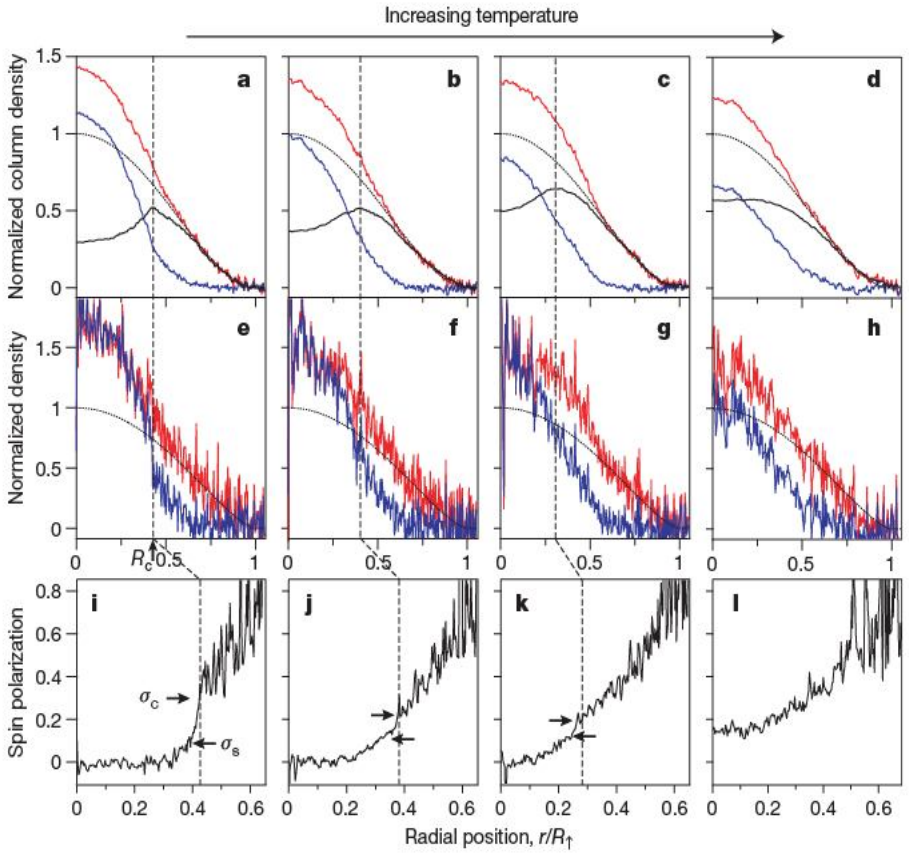


**Figure 3 | Density profiles of trapped Fermi mixtures with imbalanced populations.** The top row (**a–d**) shows the averaged column density profiles for various temperatures (red, majority; blue, minority; black, difference). The majority radius $R_\uparrow$ was determined from the outer region ($r > R_\downarrow$, where $R_\downarrow$ is the radius of the minority cloud) of the majority profiles using a fit to a zero-temperature Thomas–Fermi distribution (black dotted lines). The column densities are normalized by the central value of the fitted Thomas–Fermi distribution. The middle row (**e–h**) and the bottom row (**i–l**) show the reconstructed three-dimensional profiles and the spin polarization profiles $\sigma(r)$ corresponding to the profiles in **a–d**. The core radius $R_c$ was determined as the peak (and/or kink) position in the column density difference (only for **a–c**), indicated by the vertical dashed lines. The two spin polarizations $\sigma_c$ at $r = R_c$ and $\sigma_s$ at $r = R_c - 0.05R_\uparrow$ are marked by the right and left arrows, respectively. The values for $T'$, $\sigma_c$, $R_c/R_\uparrow$, $R_\uparrow$ (in μm), $N_\uparrow$, $\delta$ (in %) and $\lambda$ were, respectively: for **a**, **e** and **i**, 0.03(1), 0.34, 0.43, 385, $5.9(5) \times 10^6$, 44(4), 6.15; for **b**, **f** and **j**, 0.05(2), 0.24, 0.39, 416, $1.0(1) \times 10^7$, 48(4), 6.5; for **c**, **g** and **k**, 0.07(1), 0.21, 0.29, 443, $1.2(2) \times 10^7$, 54(4), 6.5; for **d**, **h** and **l**, 0.10(1), not determined, not determined ($\sigma_{r=0} = 0.15$ and condensate fraction = 2(1)%), 398, $5.3(4) \times 10^6$, 54(4), 7.7.

the superfluid at the boundary). The discontinuity in the spin polarization profile implies that there is a thermodynamically unstable window, $\sigma_s < \sigma < \sigma_c$, leading to a first-order superfluid-to-normal phase transition. As the temperature increases, the unstable region reduces with decreasing $\sigma_c$ and increasing $\sigma_s$. For high temperature when the bimodal feature in the spin polarization profile disappears, we recorded the condensate fraction as an indicator of superfluidity, using the rapid field-ramp technique[17]. As the temperature decreases, the condensate fraction gradually increases with a finite central polarization[19]. Such smooth variations of the density profile and condensate fraction across the phase transition are characteristic of a second-order phase transition.

The phase diagram is characterized by three distinct points: the critical temperature $T_{c0}$ for a balanced mixture, the critical polarization $\sigma_{c0}$ of a normal gas at zero temperature, and the tricritical point $(\sigma_{tc}, T_{tc})$ at which the nature of the phase transition changes. Owing to the lack of a predicted functional form for the phase transition line in the $\sigma$–$T$ plane, we apply a linear fit to the measured critical points, suggesting $T_{c0}/T_{F\uparrow} \approx 0.15$, $\sigma_{c0} \approx 0.36$ and $(\sigma_{tc}, T_{tc}/T_{F\uparrow}) \approx (0.20, 0.07)$. The value for $\sigma_{c0}$ agrees well with the prediction (from the quantum Monte Carlo calculation) of 0.39 (ref. 10). The extrapolation of the phase diagram to $\sigma = 0$ is tentative, because the *in situ* thermometry could not be applied to small population imbalances owing to the narrowness of the non-interacting outer region.

The Chandrasekhar–Clogston limit reflects the energetic competition between a superfluid state and a partially polarized normal state, and occurs at a critical value of $2h_c$ for the chemical potential difference $\delta\mu = \mu_\uparrow - \mu_\downarrow$. In Bardeen–Cooper–Schrieffer theory, which is valid for weak interactions, $h_c = \Delta/\sqrt{2}$ (ref. 3). Here, $\Delta$ is the pairing gap. With the assumption of no interactions in a normal gas, quantum Monte Carlo studies predict $h_c = 1.00(5)\Delta \approx 1.2\mu$ at unitarity[11], where $\mu = (\mu_\uparrow + \mu_\downarrow)/2$. The condition $\mu_{\downarrow c} = \mu - h_c < 0$ requires $n_\downarrow = 0$ for a non-interacting normal gas, implying the absence of a partially polarized normal phase and consequently $\sigma_{c0} = 100\%$. Mean-field approaches[12–16], which cannot treat the interactions in the normal phase accurately, also predict a high critical imbalance $\sigma_{c0} > 90\%$. Strong interactions in the normal phase, however, have been observed through the compressed shape of the minority cloud[18] and the shift in the radio frequency excitation spectrum[26]. The data in Fig. 5 clearly establish a zero-temperature Chandrasekhar–Clogston limit for $\sigma_{c0}$ in the range 30% to 40%. By analysing the *in situ* density profiles[25,27], we obtained $h_c \approx 0.95\mu$ (see Methods). Since theory clearly predicts $\mu < \Delta$ at unitarity[9,11], we have $h_c < \Delta$. If $h_c$ were larger than $\Delta$, polarized quasi-particles would have negative energies and would already form at zero temperature. Therefore, up to our observed value of $h_c$, the fully paired

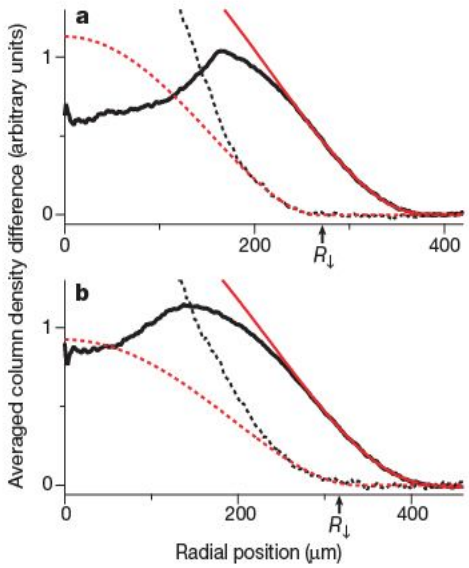


**Figure 4 | Temperature determination using *in situ* density profiles.** The relative temperature $T' = T/T_{F0}$ (see text for definition) was determined from the outer region ($r > R_\downarrow$) of the averaged column density difference profile (black line) fitted to a finite temperature Fermi–Dirac distribution (red line). The radius of the minority cloud $R_\downarrow$ was determined from a fit of the wing profile of the minority component (black dashed line) to a zero-temperature Thomas–Fermi distribution (red dashed line). **a**, $T' = 0.03(1)$ and $\delta = 44(4)\%$. **b**, $T' = 0.08(1)$ and $\delta = 46(4)\%$.

superfluid state is stable, and a polarized superfluid exists only at finite temperature.

The interface between two immiscible fluids involves a surface energy, leading to at least a small violation of the local density approximation. However, the observed sharp interface along an equipotential line and the flat-top structure of the linear density difference profiles (Fig. 2d and e) imply that corrections to the local density approximation are smaller than the resolution of our experiment. These observations are inconsistent with the interpretations given for the experimental results reported in refs 20 and 21, where it has been shown that highly elongated small samples are deformed by surface tension[28,29]. The scaling of those surface effects to our parameters predicted a deviation of the aspect ratio of the superfluid core of about 15% from the trap aspect ratio[29], whereas our data gives an upper bound of 2%. We note that surface tension would add energy in the phase-separated superfluid regime and would shift the Chandrasekhar–Clogston limit to smaller values. Refs 20 and 21 concluded that the Chandrasekhar–Clogston limit should be $\delta_{c0} > 95\%$, which is ruled out by our observations. We are not aware of any suggested effect that can reconcile the data of refs 20 and 21 with our phase diagram for a resonant superfluid. To identify this finite size effect and to understand fully the nature of the normal state[26], more work on imbalanced Fermi gases is needed.

In conclusion, we have established the phase diagram of a homogeneous spin-polarized Fermi gas with resonant interactions in the $\sigma$–$T$ plane. This includes the identification of a tricritical point at which the critical lines for first-order and second-order phase transitions meet, and the final confirmation of a zero-temperature quantum phase transition—the Chandrasekhar–Clogston limit of superfluidity—for a gas at unitarity. So far, predicted exotic superfluid states such as the breached-pair state in a stronger coupling regime (Bose–Einstein condensate side)[13] and the Fulde–Ferrell–Larkin–Ovchinnikov state in a weaker coupling regime (Bardeen–Cooper–Schrieffer side)[5,6,12,16,30] have not been observed, but the novel methods of tomography and thermometry will be important tools in the search for those states.

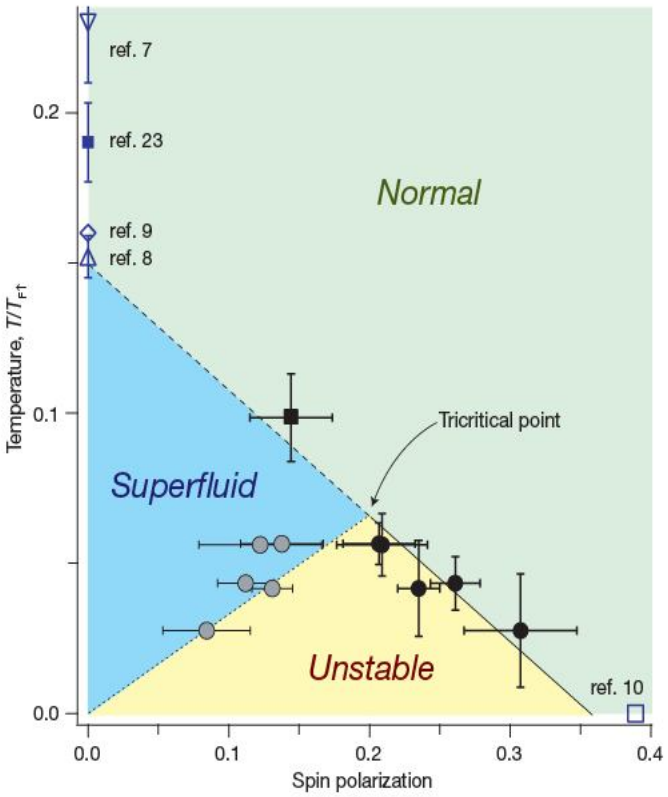


**Figure 5 | The $\sigma$–$T$ phase diagram for a homogeneous spin-polarized Fermi gas with resonant interactions.** The critical polarizations $\sigma_c$ (black solid circles and square) and $\sigma_s$ (grey solid circles) are displayed along the local $T/T_{F\uparrow}$ at the phase boundary. The yellow area ($\sigma_s < \sigma < \sigma_c$) represents a thermodynamically unstable region, leading to the phase separation. Above the tricritical point, the phase transition in the centre of the cloud was observed by the onset of pair condensation. For this, a cloud was evaporatively cooled, until it crossed the phase transition on a trajectory almost perpendicular to the phase transition line (see Supplementary Information). The critical spin polarization and temperature were obtained by interpolating between points without and with small condensates (black solid square). The linear fit to the $\sigma_c$ values is shown as a guide to the eye for the normal-to-superfluid phase transition line. Each data point consists of five independent measurements and error bars indicate standard deviation. The blue open symbols show theoretical predictions for the critical temperature of a homogeneous equal mixture[7–9] and the critical polarization at zero temperature[10]. The blue solid square is the measured critical temperature of ref. 23, multiplied by $\sqrt{\xi}$ with $\xi = 0.42$ (ref. 11) to obtain local $T/T_F$ at the centre. Finite temperature correction may increase the effective value of $\xi$.

## METHODS SUMMARY

The experimental procedure has been described in our previous publications[17–19]. A degenerate Fermi gas of $^6$Li atoms was first prepared in an optical trap, using laser cooling and sympathetic cooling with $^{23}$Na atoms. A variable spin mixture of the two lowest hyperfine states $|\uparrow\rangle$ and $|\downarrow\rangle$ (corresponding to the $|F=1/2, m_F=1/2\rangle$ and $|F=1/2, m_F=-1/2\rangle$ states at low magnetic field) was created at a magnetic field $B = 885$ G. The final evaporative cooling was achieved by lowering the trap depth and all measurements were performed at $B = 833$ G. The temperature of the cloud was controlled by the lowest value of the trap depth in the evaporative cooling process. The oscillation frequency in the axial direction was $f_z = 23$ Hz. The two transverse oscillation frequencies $f_\rho$ are equal to within less than 2%. Two phase-contrast images of the same sample were taken consecutively with different probe frequencies, $\nu_1$ and $\nu_2$ (Fig. 2). The time interval between the two images was 10 μs, and the pulse duration of each probe beam was 15 μs. Because the probe beam was off-resonant, no heating effect of the first pulse was observed in the second image. The trapped sample was observed to have an elliptical shell structure of the same aspect ratio $\lambda = f_\rho/f_z$ as the trapping potential over our entire temperature range, and we obtained the low-noise profiles $\tilde{n}$ by averaging the column density distribution along the equipotential line defined as $\lambda^2 x^2 + z^2 = r^2$ for a given radial position $r$. The region for averaging was restricted depending on the type of analysis. Deviations from the trap aspect ratio were only found for the outer thermal wings. Details of the phase-contrast imaging technique and the data analysis are given in Methods and Supplementary Information.

**Full Methods** and any associated references are available in the online version of the paper at www.nature.com/nature.

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LETTERS

# An updatable holographic three-dimensional display

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**Holographic three-dimensional (3D) displays[1,2] provide realistic images without the need for special eyewear, making them valuable tools for applications that require situational awareness, such as medical, industrial and military imaging. Currently commercially available holographic 3D displays[3] use photopolymers that lack image-updating capability, resulting in restricted use and high cost. Photorefractive polymers[4–9] are dynamic holographic recording materials that allow updating of images and have a wide range of applications, including optical correlation[10], imaging through scattering media[11] and optical communication[12,13]. To be suitable for 3D displays, photorefractive polymers need to have nearly 100% diffraction efficiency, fast writing time, hours of image persistence, rapid erasure, and large area—a combination of properties that has not been shown before. Here, we report an updatable holographic 3D display based on photorefractive polymers with such properties, capable of recording and displaying new images every few minutes. This is the largest photorefractive 3D display to date (4 × 4 inches in size); it can be recorded within a few minutes, viewed for several hours without the need for refreshing, and can be completely erased and updated with new images when desired.**

A considerable amount of research has been dedicated to the development of 3D imaging[14–22], because two-dimensional (2D) images give only limited information about an object or a scene owing to their lack of parallax and depth[17]. 3D imaging techniques that rely on special eyewear have unwanted side-effects such as eye fatigue and motion sickness. Holographic 3D displays do not incur these problems because they are viewable with the naked eye (autostereoscopic) and simulate natural human vision. Humans are naturally attracted to holograms, which is why holography has found wide applications in advertisement and entertainment. Current static holographic displays[3] are capable of displaying terabytes of data, and come in practically any size with full colour, full parallax and depth. Previously, dynamic 3D holographic displays based on acousto-optic[23], liquid-crystal displays[24] and microelectromechanical-systems-based recording media[25] have been demonstrated. Unfortunately, these devices do not have memory, and thus do not exhibit persistence of recorded images. The lack of persistence results in the requirement of update rates faster than 30 Hz to avoid image flicker. 3D images exhibit very high information content, so this high refresh rate requirement currently limits real-time holographic displays to small sizes. Photorefractive inorganic crystals are dynamic holographic storage materials that have memory[26], but scaling them to the large sizes needed for 3D displays is challenging. Photothermoplastics provide reversible recording by using surface relief gratings, but they suffer from limited diffraction efficiency and usually require a post-recording developing process. To extend dynamic holographic 3D displays towards practical applications, alternative materials with high efficiency, reversible recording capabilities, memory and significantly larger sizes are needed.

Photorefractive polymers are dynamic holographic recording materials capable of fulfilling these requirements[4–13]. In photorefractive polymers, a 3D refractive index pattern—a phase hologram—replicates the non-uniform interference pattern formed by two incident coherent light fields. This effect is based on the build-up of an internal space-charge field due to selective transport and trapping of the photo-generated charges, and an electric-field-induced index change via the photorefractive effect (ref. 5 and references therein and ref. 4). This process—in contrast to the photochemical processes involved in photopolymer holograms—is fully reversible, because trapped charges can be de-trapped by uniform illumination. The erasibility of the photorefractive gratings allows for refreshing/updating of the holograms. In a typical photorefractive material the holograms are viewed with the help of a reading beam, as long as the initial writing (recording) beams are present. When the writing beams are turned off, the photorefractive hologram decays at a rate determined by the material properties and ambient thermal processes. Photorefractive polymers that have fast recording usually have high decay rates. For updatable 3D displays, however, a material with rapid recording and slow decay (long persistence) is required. A figure-of-merit (FOM) for the design of recording media for spatially multiplexed 3D displays can be the ratio of storage time to the total recording time during which the writing beams are turned on, per holographic element (hogel). In most photorefractive materials the FOM is close to 1, which is far smaller than the FOM $>$ 1,000 required for use in updatable 3D displays with large enough size and resolution.

We have developed photorefractive polymer composites that combine these properties, suitable for use in updatable 3D displays. The composite consists of a copolymer with a hole-transporting moiety and a carbaldehyde aniline group (CAAN) attached through an alkoxy linker. The copolymer approach is adopted to minimize the phase separation between the functional components usually seen in homopolymer photorefractive composites. A copolymer with a polyacrylic backbone was used to attach pendant groups, tetraphenyldiaminobiphenyl-type (TPD) and CAAN in the ratio 10:1 by the synthetic modification of the polyacrylate TPD (PATPD) polymer[12]. The host PATPD-CAAN copolymer provides the optical absorption and charge generation/transport at the writing wavelength (532 nm). A plasticizer, 9-ethyl carbazole (ECZ) was added to the composite. The non-linear optical (NLO) properties were achieved by adding a fluorinated dicyanostyrene (FDCST) chromophore. The composite PATPD-CAAN:FDCST:ECZ (50:30:20 wt%) was formed into thin-film devices by melting it between two indium-tin-oxide-coated glass electrodes with a thickness of 100 μm set by glass spacer beads. This composite showed no phase separation in an accelerated ageing test

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at 60 °C for 7 days. The photorefractive thin-film devices show near 90% diffraction efficiency at an applied voltage of 4 kV in steady-state four-wave mixing measurements (Fig. 1a). The two-beam coupling gain coefficient $\Gamma$ for these devices at 5 kV is around 200 $cm^{-1}$ (inset to Fig. 1a). Figure 1b shows a 4 × 4-inch active area thin-film device made from this composite. The device shows no degradation or dielectric breakdown for extended periods of usage (several months) in display recording experiments, with hundreds of write/erase cycles every month at high applied voltages (9 kV) and optical intensities around 100 mW $cm^{-2}$.

The holograms recorded in the photorefractive polymer thin-film devices can persist for up to 3 h in the dark (without writing beams) at an applied voltage of 4 kV, while continuously being probed with a red (633 nm) laser beam. We have developed a new technique to improve the writing speed of organic photorefractive materials that is based on manipulation of the applied voltage, which we call the 'voltage kick-off technique'. In conventional holographic recording of photorefractive polymers, a constant external voltage is applied across the polymer to dynamically pole the NLO chromophores (ref. 5 and references therein and ref. 4). In the kick-off approach, we apply an increased voltage (9 kV) across the polymer to increase the writing speed during hologram recording, and then reduce the voltage to its optimum value of 4 kV after recording is complete. The temporarily increased voltage facilitates efficient separation of electron–hole pairs, and improves the drift characteristics, forcing the charges to travel faster, and increases the orientational order parameter and speed of the NLO chromophores. The reduction of the voltage to its optimum value after recording ensures hologram persistency. The overall benefit of the voltage kick-off is the reduction of the writing time per hogel to less than a second, by fine-tuning of the applied voltage. We have achieved a diffraction efficiency of 55% using a total writing time (the time during which the writing beams are turned on) of only 0.5 s at 1 W $cm^{-2}$ irradiance with this technique (Fig. 2), much higher than the 1.5% efficiency achieved with writing for 0.5 s at 4 kV without using voltage kick-off. With the several hours of persistence time of holograms in this composite, this corresponds to a FOM > 10,000 without the need for thermal[27] or other fixing methods, which is a significant step in the development of photorefractive polymers for holographic storage and display applications.

Holographic stereography—a technique based on optical multiplexing of a limited number of viewpoints (perspectives) onto different parts of a recording medium—is a widely used technique for producing 3D imagery and displays[14,28,29]. We have built a fully automated, computer-controlled 3D holographic printer/display based on holographic stereography using the photorefractive polymer devices described above. The 3D display is recorded onto the entire photorefractive polymer device with an active area of 4 × 4 inches (Fig. 3). First, 2D perspective views of the object of interest are generated from a 3D computer model. The 2D perspectives can also be generated using methods like magnetic resonance imaging,

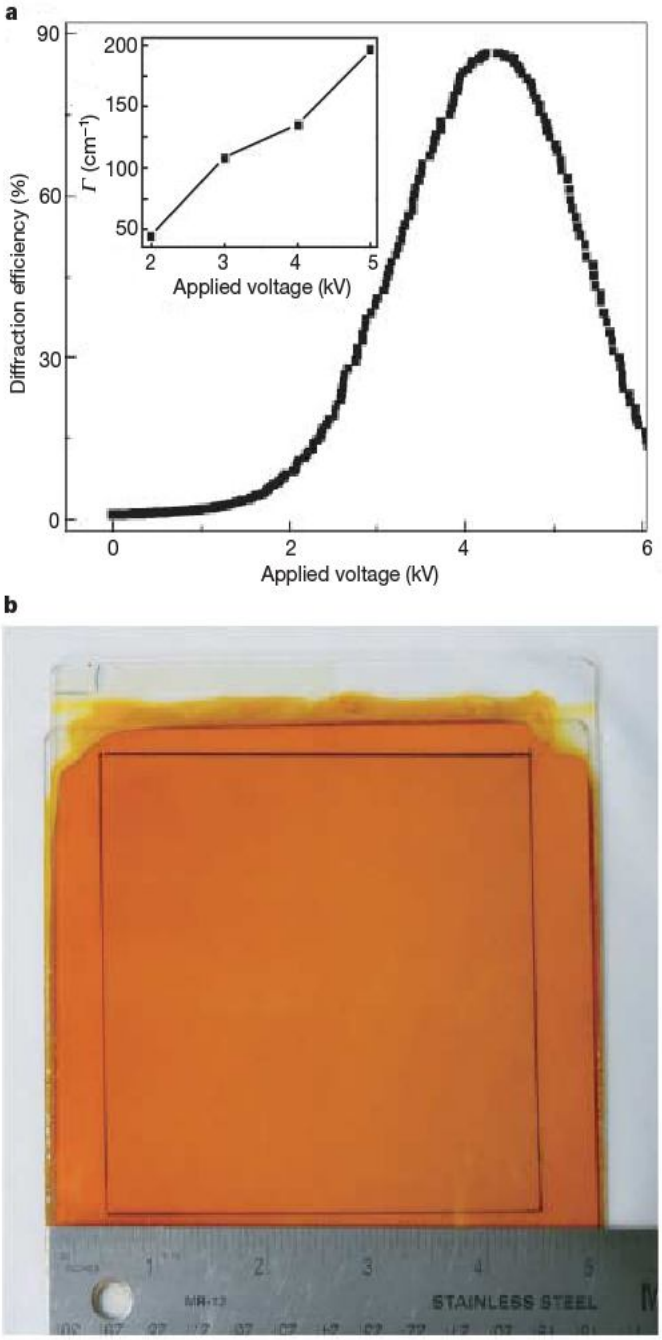


**Figure 1 | Diffraction efficiency and the photorefractive polymer thin-film device. a**, The steady-state diffraction efficiency of the 100-μm-thick polymer composite is measured using writing beams at 532 nm with a total irradiation of 1 W $cm^{-2}$ and a reading beam at 633 nm. Inset, two-beam coupling gain versus applied voltage. **b**, Picture of a 4 × 4-inch photorefractive polymer thin-film device.

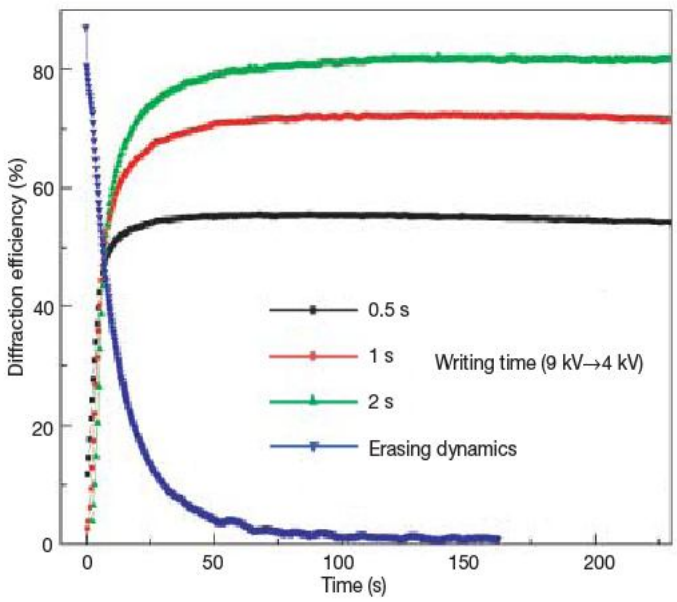


**Figure 2 | Recording dynamics of the photorefractive polymer and voltage kick-off.** The writing beams at 532 nm with 1 W $cm^{-2}$ irradiance are turned on at an applied voltage of 9 kV for a few seconds (writing time), and then turned off. The voltage is then reduced to 4 kV to ensure hologram persistence and high diffraction efficiency. The maximum diffraction efficiency achieved increases with increasing writing time. A modified version of this technique was used for the 3D display recording of Fig. 4. Also shown is the erasing dynamics of the photorefractive hologram at 9 kV. The erasing beam is a spatially uniform 532 nm laser beam at an irradiance of 1 W $cm^{-2}$.

computer-assisted tomography, confocal microscopy or aerial and satellite imaging. The perspectives are then divided or 'sliced' into multiple 2D image planes. The image planes are re-organized using a computer algorithm into 2D matrices (the hogel data), which are then uploaded to a spatial light modulator (SLM). The SLM that is illuminated with a 532 nm laser beam displays the hogel data in sequence with the translation stages and an electro-optic laser shutter. The laser beam modulated by the SLM (object beam) illuminates the predefined hogel area on the polymer device. A coherent reference beam simultaneously illuminates the same area, which facilitates the recording of the hogel through interference with the object beam and the photorefractive effect. After one hogel is recorded the shutter turns off the laser beams, the polymer device is translated to the next hogel position, and new hogel data are uploaded to the SLM (see Supplementary Video 1 for display operation). The holographic display is viewed using light from an expanded, low-power helium neon (633 nm) laser beam in transmission geometry (Fig. 3).

In many applications, horizontal parallax only (HPO) imaging[29] is an effective approximation to full-parallax imaging, because humans perceive depth using horizontally offset eyes. The use of HPO recording helps in significantly reducing the number of hogels in a 3D display, resulting in shorter total writing times. We have recorded 3D displays ($4 \times 4$ inches in size) with complex and high-quality images (Fig. 4) within a few minutes using HPO imaging (see Supplementary Video 2). The total recording time used per hogel ($0.8 \times 101$ mm in size) varies from 0.5 to 2 s, and the total irradiance (sum of both beams) used is 0.1 W cm$^{-2}$.

Here, we used a modified version of the voltage kick-off technique, in which a constant high voltage (9 kV) was applied to the entire polymer device during recording of the hogels. Once recording of all of the hogels is completed, which takes around 2.5 min, the voltage was reduced to its optimal value of 4 kV, which ensures long persistence with maximum diffraction efficiency. The first few recorded hogels suffer a small reduction of diffraction efficiency owing to the high applied voltage during recording of the later hogels, but this lower diffraction efficiency does not create a noticeable brightness variation across the display (see Supplementary Videos 1 and 2). For larger displays the variation may be significant, but this can be avoided by the use of patterned electrodes that allow for individual control of the applied voltage for each hogel.

The 3D display exhibits a total horizontal viewing angle of 45° with uniform brightness, and a resolution that is comparable to NTSC (National Television System Committee) television. The Bragg mismatch usually observed in non-degenerate four-wave mixing that results in intensity variations across the horizontal view-zone is minimized by using a vertical reference/reading beam geometry. The images are viewable for up to 3 h directly on the photorefractive thin-film device without the need for intermediate projection tools or magnification between the recorded image and the viewer (Fig. 4b).

The pictures of the holograms in Fig. 4, which were captured using a video camera, are only modest facsimiles of the effect experienced upon direct viewing. This is principally due to the astigmatism introduced by the HPO recording technique and electronic artefacts such as saturation, to which the human visual system is relatively insensitive. The images can be completely erased within minutes by uniform illumination of the display using a 532 nm beam (Fig. 4c), and new images can be recorded when desired. There is no technological limit to the achievable display size, because large thin-film devices can be fabricated and even tiled together. Moreover, the persistence and efficiency of the material make it the leading candidate for future full-parallax displays, which typically require two orders of magnitude more information content than HPO displays. For larger, full-parallax displays a combination of short pulsed recording[30] and thermal fixing[27] can be used, which is a future route for holographic 3D display development.

Image-updating capability can significantly extend the applications of holographic 3D displays and reduce the cost of 3D imaging. We have developed photorefractive polymer devices that combine exceptional properties such as large size, high efficiency, fast recording, image persistency, long lifetime and resistance to optical and electrical damage, satisfying many of the major requirements for use in holographic 3D displays. These advances have allowed us to

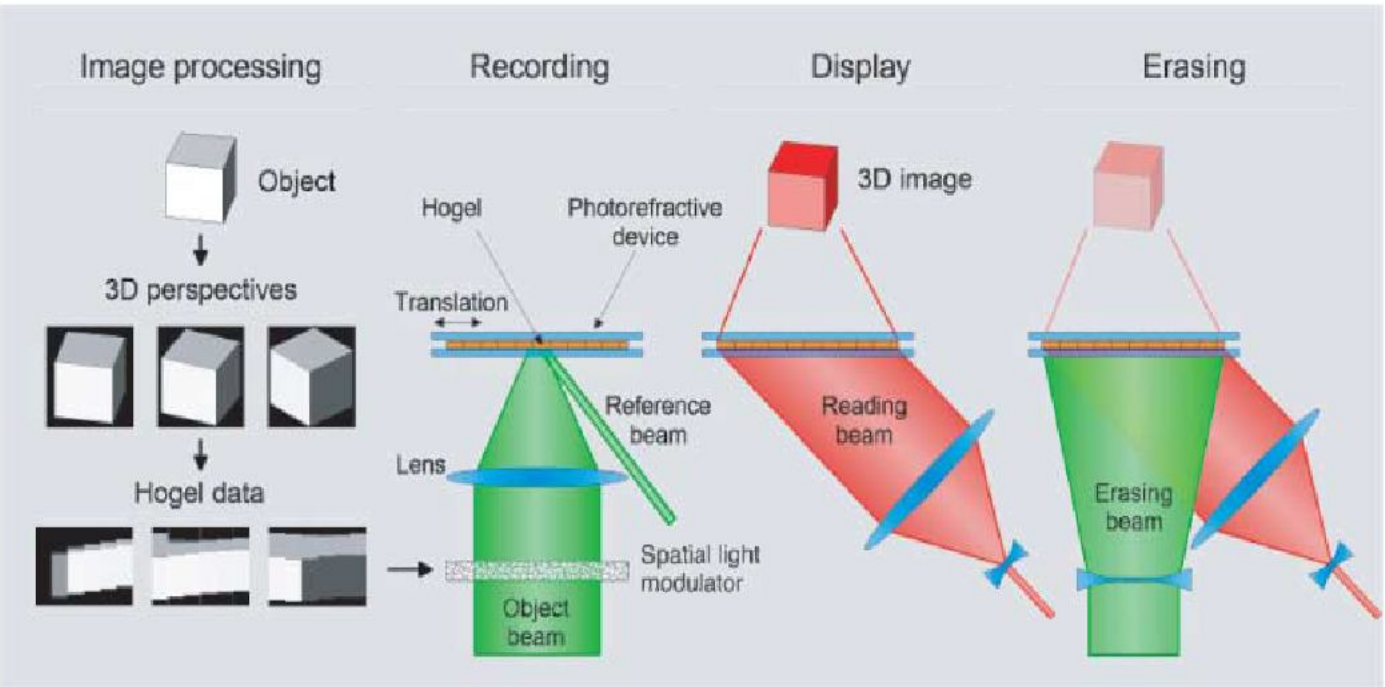


**Figure 3 | Image processing, hologram recording and display.** The 2D perspective views of the object are generated using a 3D computer model or a video camera moving on tracks around the object. The perspective images are re-organized (hogel data) and uploaded to the SLM. The SLM modulates the object beam, which is focused to the photorefractive polymer and recorded in the Fourier transform geometry. The completed display can be viewed using a reading beam. The result is realistic 3D imagery with parallax and depth. The holograms can be erased by uniform illumination at the writing wavelength.

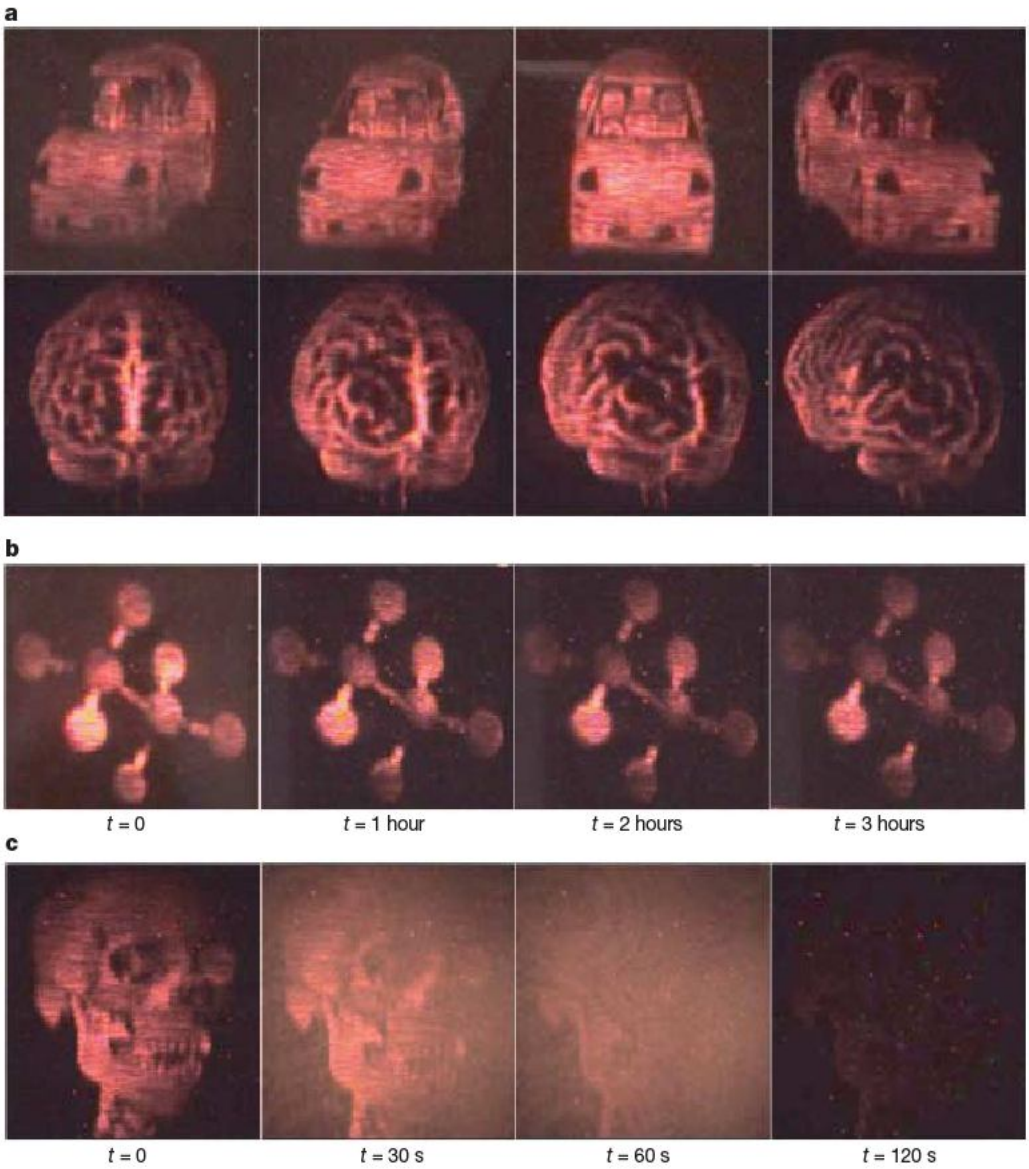


**Figure 4 | Images from the updatable holographic 3D display.** The display uses a single photorefractive thin-film device with an active area of $4 \times 4$ inches. Here, a modified version of the voltage kick-off was used to avoid using patterned electrodes. A constant voltage (9 kV) was applied across the entire polymer. Once recording of all of the hogels was completed, the voltage was reduced to its optimal value of 4 kV. **a**, A 3D hologram of a sports car was written, displayed, and then erased and a new hologram of a human brain was recorded onto the same area. The images were captured from a distance of 75 cm from different angles to demonstrate the 3D effect using a video camera moving around the display. **b**, The persistence of the hologram, in this case a 3D model of an ethane molecule, is demonstrated by capturing pictures at different times after recording. **c**, Erasure of a 3D image, a human skull, using uniform exposure is demonstrated.

demonstrate the largest updatable photorefractive holographic 3D display so far, scalable to full parallax and colour.

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LETTERS

# Geological record of fluid flow and seismogenesis along an erosive subducting plate boundary

Paola Vannucchi[1], Francesca Remitti[2] & Giuseppe Bettelli[2]

**Tectonic erosion of the overriding plate by the downgoing slab is believed to occur at half the Earth's subduction zones[1,2]. *In situ* investigation of the geological processes at active erosive margins is extremely difficult owing to the deep marine environment and the net loss of forearc crust to deeper levels in the subduction zone. Until now, a fossil erosive subduction channel—the shear zone marking the plate boundary[3]—has not been recognized in the field, so that seismic observations have provided the only information on plate boundary processes at erosive margins. Here we show that a fossil erosive margin is preserved in the Northern Apennines of Italy. It formed during the Tertiary transition from oceanic subduction to continental collision, and was preserved by the late deactivation and fossilization of the plate boundary. The outcropping erosive subduction channel is ~500 m thick. It is representative of the first 5 km of depth, with its deeper portions reaching ~150 °C. The fossil zone records several surprises. Two décollements were simultaneously active at the top and base of the subduction channel. Both deeper basal erosion and near-surface frontal erosion occurred. At shallow depths extension was a key deformation component within this erosive convergent plate boundary, and slip occurred without an observable fluid pressure cycle. At depths greater than about 3 km a fluid cycle is clearly shown by the development of veins and the alternation of fast (co-seismic) and slow (inter-seismic) slip. In the deepest portions of the outcropping subduction channel, extension is finally overprinted by compressional structures. In modern subduction zones the onset of seismic activity is believed to occur at ~150 °C, but in the fossil channel the onset occurred at cooler palaeo-temperatures.**

Tectonic erosion is commonly assumed to take place within the upper plate landward of a frontal prism (Fig. 1a, b). The frontal prism at the toe of erosive subduction zones is rarely static. Originally thought to be an accretionary structure composed of sediments scraped from the incoming plate, it is now known that it is often a contractional structure composed of disaggregated material from the upper plate[4]. Deeper, tectonic erosion has been assumed to occur by high-friction mechanical coupling between the plates, inducing pervasive abrasion of the overriding plate[5,6] (Fig. 1a). In contrast, alternative hypotheses[7] and geophysical data suggest that subducting plate boundaries are fluid-rich[8,9]. Fluids may play a key part in affecting the frictional behaviour of faults[10,11] and in weakening the upper plate[4] (Fig. 1b).

Here we discuss observations from a newly recognized fossil erosive subduction channel active from late Eocene (~35 Myr ago) to middle Miocene (~11 Myr ago). These outcrops in the Northern Apennines of Italy (Fig. 2) are a unique example of an erosive subduction zone where preservation occurred because of the deactivation of the plate boundary (followed by exhumation and partial erosion) (Supplementary Note 1). Here we focus on the implications of this preserved geological record for details of erosive plate boundary structures and deformation processes.

The Apennine subduction channel is ~500 m thick and extends 200 km along-strike. It is formally known among Apennine geologists as the Sestola–Vidiciatico unit (SVU) (Fig. 2). At present, the SVU is sandwiched between the overlying Late Cretaceous/early Eocene accretionary prism (that is, the frontal European plate margin) and the underlying fold-and-thrust belt formed by Adriatic continental units. The subduction channel is made of a melange formed by tectonically and gravitationally reworked blocks of (1) the previous, Late Cretaceous/early Eocene accretionary prism, (2) debris flow deposits of the frontal prism, and (3) late Eocene/middle Miocene slope sediments deposited on top of the frontal prism[12] (Supplementary Note 2).

Thus the SVU represents a large shear zone between the overriding European plate and the underlying Adriatic plate, and it contains material coming only from the overriding plate, consistent with it being the shallow portion of an erosive subduction channel.

The poorly exposed upper tectonic contact of the SVU with the overlying fossil accretionary prism maintains a planar geometry at the regional scale, indicating that, although active, it has never been involved in the collisional fold-and-thrust architecture[13] (Fig. 2).

The well-exposed lower tectonic contact of the SVU on the foredeep sequences has a map-scale ramp-and-flat geometry[14] with the deepest outcropping portion involved in a series of kilometre-size thrusts and folds[13] (Figs 2, 3). The development of these shortening structures indicates the deactivation of the deeper portion of the basal décollement, whereas its shallower portion and the upper décollement were still active and responsible for the northeastward migration of the upper plate on top of the foredeep turbidites. We propose that deactivation of the deeper portion of the lower décollement is associated with a period of uplift during the early–middle Miocene boundary[15,16] that reflects crustal thickening by tectonic underplating of subducted material along the landward portion of the forearc at the same time as tectonic erosion was occurring close to the trench.

The presence of a roof and a basal décollement that simultaneously cut through the margin toe implies that they were able to incorporate intact slices of the frontal prism through a process of frontal tectonic erosion (Fig. 1c). This ongoing process progressively incorporated younger slope sediment and older parts of the overlying former accretionary prism.

Our study of the internal fabric of the subduction channel concentrated on the slope sediment blocks that do not contain the deformation imprint of any previous subduction phase. In the shallower portion of the subduction channel (less than ~3 km, based on the thickness of overlying sediments) these blocks record a pervasive deformation represented by sets of extensional shear fractures

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(Fig. 3a) with minor contraction. Veining is absent. The fractures tend to die out along the contact with the already lithified pieces of the melange, indicating strong differences in mechanical behaviour between its components. The field data sets (Fig. 2) consistently show two conjugate and contemporaneous sets of gently dipping normal faults producing a three-dimensional (3D) geometry comparable to a flattened bipyramid (Fig. 3a). The bipyramids have their minor axis nearly parallel to the vertical direction where the faces show angles of 110–120°. The observed polymodal fracture pattern can be explained by a 3D brittle shear failure criterion[17,18]. However, this model fails to explain the development of two conjugate shear planes that are inclined at an angle >45° to the axis of maximum compressive stress, although a high angle is consistent with unconsolidated, hence very weak sediments. Apparently, this shallower portion accommodated strain through contemporaneous failure and compaction, the latter inducing flattening of the bipyramids.

We speculate that consolidation is difficult in a fluid-rich, low-permeability environment. Strengthening may occur through the development of high fracture densities that act as dewatering conduits[19]. The sediment volume then becomes separated into multiple discontinuous elements along the subduction channel. The deformation zone remains weak because its elements are continually cut by new fracture episodes as soon as they develop strain hardening. In addition, this shallower portion of the subduction channel, in spite of being a mega-shear zone in a compressive geodynamic environment, experienced simultaneous extension.

In the intermediate portion of the subduction channel, evidence of extension is also found where diffuse deformation evolves into concentrated shear. Here, normal faults formed with a spacing of ~10 m. They cut the basal décollement (Fig. 3b), often reusing pre-existing discontinuities, and they are cut by successive slip along the décollement. These structures intersect all the SVU components, indicating that by this stage the unit had a homogeneous mechanical behaviour. Clay mineral assemblages are seen to have undergone a partial to total smectite-to-illite transformation; this reaction implies an important fluid source (Fig. 1d). Discrete normal faults also developed in the

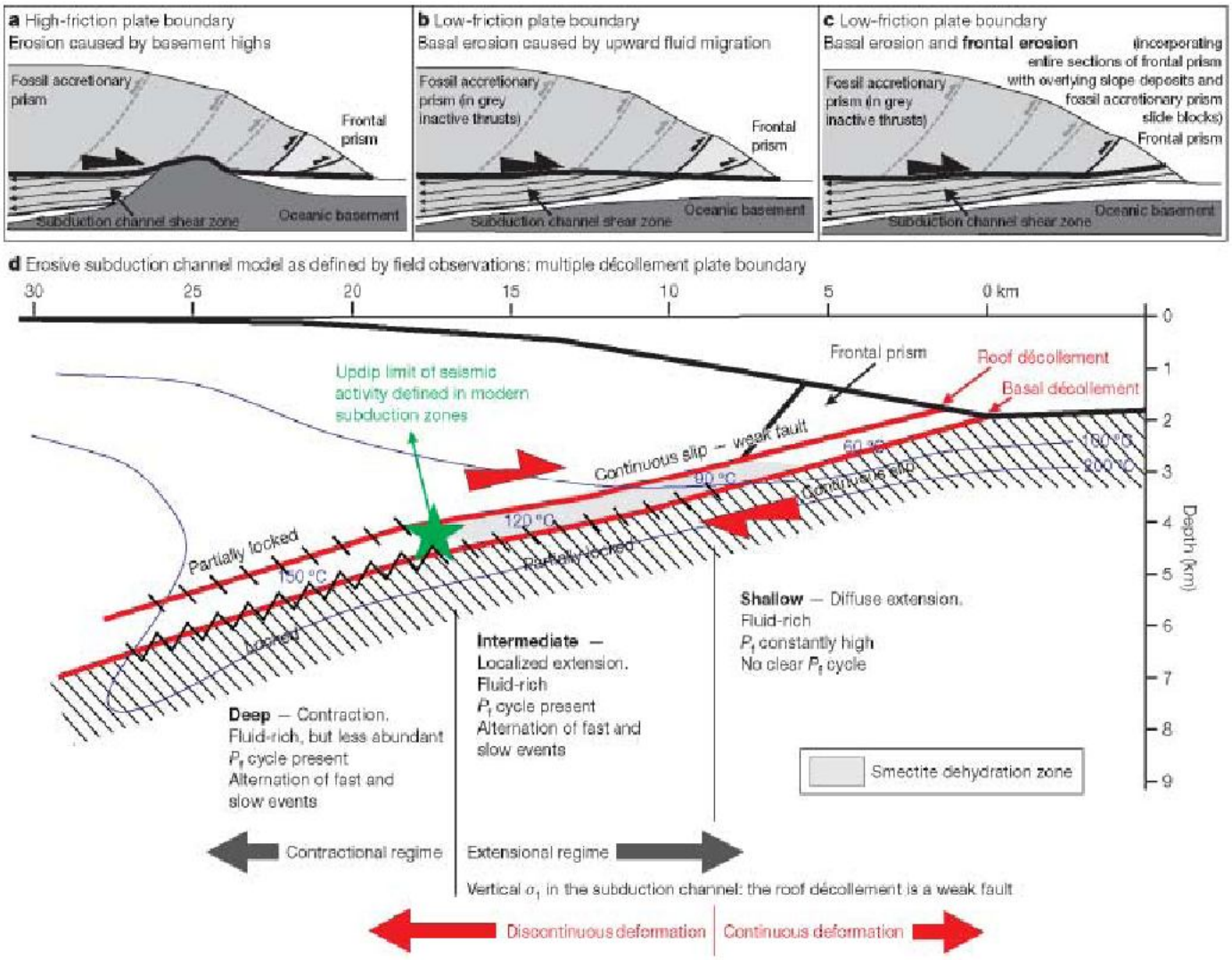


**Figure 1 | Schematic models of tectonic erosion.** Arrows in the subduction channel indicate relative velocities removing material from the base of the overriding plate. **a**, The model used in refs 5, 6 and 27: high-friction décollement steps up into a fossil accretionary prism as a result of subduction of the basement high (inactive thrusts are shown in grey). The subduction channel incorporates material ahead of the basement high. **b**, The model used in ref. 4: low-friction décollement steps up as a result of high fluid pressure causing hydrofracturing and weakening at the base of the overriding plate. Tectonic erosion is localized landward of the frontal prism and the subduction channel incorporates blocks from the overriding plate. **c**, The model proposed in this paper: a low-friction décollement cuts through the margin toe and incorporates in the subduction channel debris material from the frontal prism and associated slope sediments as well as material from the base of the overriding plate. **d**, Schematic model of the Apennine subduction channel. Thermal constraints and seismic activity[28,29] are taken from the well-studied erosive Costa Rica margin. The SVU subduction channel indicates an abundance of fluids throughout its length, but with different fluid sources. In the shallow portion the unconsolidated state of the sediment suggests that the predominant fluid source was sedimentary water. In the intermediate portion, consolidation suggests that sedimentary water is no longer the predominant fluid source. Clay mineral assemblages record a partial to total smectite-to-illite transformation, so that here this reaction could have been the predominant fluid source[25]. For the basal décollement, evidence for discontinuous slip occurs at shallower depth than for the roof décollement, and it migrates landward as well as upward. Evidence for discontinuous slip is even present in the extensional strain regime of the subduction channel.

footwall foredeep turbidites, but these footwall faults are widely spaced and only through the first 10 m below the contact. Faults are 10 cm to 1 m thick and permeated by dense arrays of extensional calcite veins. Each fault accommodates centimetre-scale displacement that is reached after a great number of repeated smaller events. The internal structure of the veins indicates that the faults were initially characterized by dilational stepovers, which allowed the opening of void spaces, and crack-and-seal fabric and pressure-solution testify to cyclical fluid pressure rises and drops that were followed by mineral precipitation, and then a new loading phase (Fig. 3b). Brecciation of both the previously precipitated calcite and the wall rocks is present within the dilational jogs, suggesting a triggering mechanism related to local fluid pressure drops, that is, implosions.

Strain concentration within the subduction channel developed in a generally extensional regime, associated with a fluid pressure cycle[20,21]. Two competing processes accommodate this deformation: (1) calcite veins and implosion breccias indicating relatively fast events and stress drop, and (2) slower pressure-solution.

In the deeper portion of the outcropping subduction channel extensional structures are finally overprinted by contractional structures. Here clay mineral assemblages, fluid inclusions, reflectance of organic matter[22], and fission tracks[23] all indicate that the sediment reached ~150 °C, corresponding to a depth of ~5 km. In modern subduction zones, 150 °C is often thought to be a key threshold temperature marking the updip limit of seismogenesis[24,25]. The involvement of the basal décollement in the fold-and-thrust deformation indicates locking, although fluid circulation was still active as shown by vein development (Fig. 3c).

Focusing on the relationship between the onset of shear concentration and the seismic cycle, the SVU points to a key role for episodes

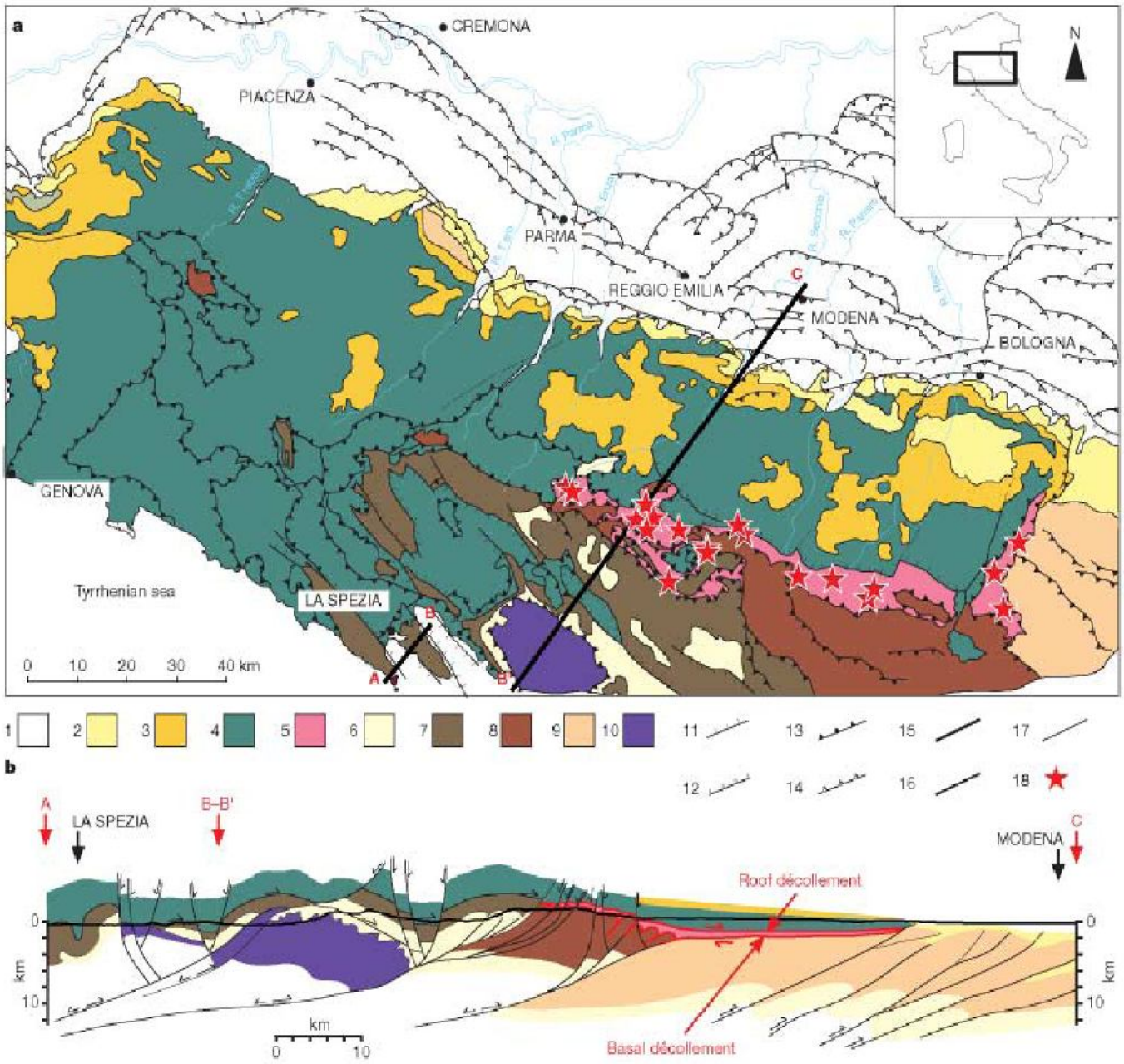


**Figure 2 | Geological setting of the Northern Apennines.** **a**, Schematic geological map with its geographic location shown in the inset. Key: (1) Quaternary deposits; (2) late Miocene–Pleistocene marine deposits; (3) Forearc slope deposits; (4) Oceanic units of the Late Cretaceous/early Eocene accretionary prism (European plate); (5) Sestola–Vidiciatico tectonic unit (subduction channel); (6) Mesozoic carbonate units of the Adria plate; (7) late Oligocene/early Miocene (Aquitanian) trench turbitites of the Adria plate; (8) early Miocene (Aquitanian–Burdigalian) foredeep turbidites of the Adria plate; (9) middle Miocene/late Miocene (Langhian–Messinian) foredeep turbidites of the Adria plate; (10) metamorphic continental units of the Adria plate; (11) normal faults; (12) normal faults (subsurface); (13) thrust faults and overthrusts; (14) thrust faults (subsurface); (15) strike-slip faults; (16) high-angle faults of unknown displacement (subsurface); (17) lithological boundaries; (18) location of detailed studied structural sections. **b**, Geological cross-section as marked on map (A–C). The thickness of the SVU is about 500 m, slightly decreasing towards the northeast.


**Figure 3 | Photographs of mesoscopic structures characterizing the Apennine fossil subduction channel.** **a**, Shallow portion: (1) and (2), extensional shear fractures cutting through the blocks of lower slope marls at the metre-scale and at the centimetre-scale. Red arrows indicate the fractures. The shear fractures occur at all scales of observations in the same geometry, until the complete loss of the sedimentary fabric. (3) Equal-angle, lower-hemisphere stereographic projection of extensional shear fractures and associated striae at the site shown in (1). (4) Three-dimensional bipyramidal element defined by the extensional shear fractures. **b**, Intermediate portion: (5) Basal décollement separating the SVU/subduction channel from the underlying foredeep turbidites of the Adria plate. Here the basal décollement (~5 cm thick) is separating the debris flow component of the subduction channel from the foredeep turbidites. The basal décollement is cut by high-angle normal faults filled by calcite veins and with displacements of about 1–2 m towards the southwest. In general the main direction of movement recorded by the normal faults is parallel to the direction of the Apennine tectonic transport, northeast, but southwest, northwest and southeast displacements are also present. (6) Close up of the shear zone marking the basal décollement; (7) dilational jog along the basal décollement; (8) thin section of a calcite vein showing crack-and-seal fabric (parallel polars). **c**, Deep portion: (9) basal décollement involved in an anticline fold indicating shortening and locking; (10) the basal décollement, which now has a vertical orientation as a consequence of its involvement in an anticline, shows extensional calcite veins associated with the shortening stage of the basal décollement.

of extensional strain, where recurrent normal faulting creates a structural environment in which hydrofracturing can occur at relatively low values of $\lambda_v = P_f/\sigma_v$ (where $P_f$ is fluid pressure and $\sigma_v$ is overburden pressure)[26]. In this system we envisage conditions of anomalous high sediment strengthening relative to depth of the subduction channel.

The geological record within the fossil SVU subduction channel provides the following constraints on the tectonics and fluid flow at erosive subduction margins. (1) Two décollements were simultaneously active during plate convergence. They show differing down-dip mechanical slip behaviour and fluid flow. The presence of extensional shear strain in the channel and in foredeep turbidites indicates that the décollements were able to transmit lithostatic loads. This implies their weak nature until at least intermediate (~3 km) depths where the basal décollement became partially locked. At ~5 km depths, the basal décollement became fully locked, but the roof décollement was still able to transmit lithostatic load to the intermediate portion of the channel and became partially locked only in the deeper portion (Fig. 1d). (2) Both deeper basal erosion and near surface frontal erosion occurred. Frontal erosion has not yet been recognized at modern subducting margins, but may provide a key to interpret seismic images. (3) At shallow to intermediate depths, transient phases of extension with a large pure-shear component are a key mode of deformation within this convergent plate boundary. (4) At shallow depths (less than ~3 km), slip occurred without an observable fluid pressure cycle. We believe that this happened because the rock was too soft to let fluid pressure build up. Somewhat deeper in the subduction channel, as rocks acquired more strength, a fluid cycle is clearly shown by the development of veins. This fluid cycle records alternation of fast (co-seismic) and slow (inter-seismic) slip. Even deeper there is no evidence for convergence-linked extension, but the same fast and slow slip events are indicated within the subduction channel. (5) This subduction channel records the same 150 °C temperature that correlates with the updip limit of seismogenesis at modern subduction zones. Here the aseismic–seismic transition may correspond to the onset of the fluid pressure cycle in the extensional regime at temperatures even lower than 150 °C, with 150 °C marking the transition from extensional to compressional shear. This conjecture will be testable at modern subduction zones by drilling currently planned as part of the Integrated Ocean Drilling Program.

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**Supplementary Information** is linked to the online version of the paper at www.nature.com/nature.

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LETTERS

# Palaeotemperature trend for Precambrian life inferred from resurrected proteins

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**Biosignatures and structures in the geological record indicate that microbial life has inhabited Earth for the past 3.5 billion years or so[1,2]. Research in the physical sciences has been able to generate statements about the ancient environment that hosted this life[3–6]. These include the chemical compositions and temperatures of the early ocean and atmosphere. Only recently have the natural sciences been able to provide experimental results describing the environments of ancient life. Our previous work with resurrected proteins indicated that ancient life lived in a hot environment[7,8]. Here we expand the timescale of resurrected proteins to provide a palaeotemperature trend of the environments that hosted life from 3.5 to 0.5 billion years ago. The thermostability of more than 25 phylogenetically dispersed ancestral elongation factors suggest that the environment supporting ancient life cooled progressively by 30 °C during that period. Here we show that our results are robust to potential statistical bias associated with the posterior distribution of inferred character states, phylogenetic ambiguity, and uncertainties in the amino-acid equilibrium frequencies used by evolutionary models. Our results are further supported by a nearly identical cooling trend for the ancient ocean as inferred from the deposition of oxygen isotopes. The convergence of results from natural and physical sciences suggest that ancient life has continually adapted to changes in environmental temperatures throughout its evolutionary history.**

Computational reconstruction and laboratory resurrection of ancestral sequences provide an opportunity to rewind the 'tape of life'. This form of time travel has been exploited to improve our understanding of the molecular adaptation of substrate specificity, the response of organisms to external stimuli, and the environmental temperatures of ancient organisms, among others (reviewed in ref. 9).

Ancestral sequence reconstruction uses standard statistical theory to generate posterior probabilities of different reconstructions given the data at a site from aligned sequences. For each site of the inferred sequence at a phylogenetic node, posterior values for all 20 amino acids are calculated and represent the probability that a particular amino acid occupied a specific site in the protein during its evolutionary history. This posterior probability distribution is calculated from patterns of amino acids in modern sequences as described by a phylogeny, a matrix of amino-acid replacement probabilities, amino-acid equilibrium (stationary) frequencies, phylogenetic branch lengths and site-specific replacement rates. The most probabilistic ancestral sequence (M-PAS) uses the amino acid with the highest posterior probability at each site within the distribution.

Despite insightful studies, the field of ancestral sequence reconstruction is encumbered by its inability to know whether inferred sequences truly recapitulate ancestral forms[10]. Practitioners in the field acknowledge a certain degree of inaccuracy associated with reconstructing ancestral sequences. The concern is not necessarily whether the resurrected form has the exact composition (genotype) of the true ancestral form, but rather that the resurrected form displays the exact behaviour (phenotype). A reconstructed sequence can be considered a consensus of a gene distributed throughout a population before species divergence or before gene duplication. Inaccuracies in a reconstructed sequence can result from sequence variation in the gene itself within an ancient population. If one assumes that the variants of a homologous gene within a population had the same phenotype at a specific geological time, it does not necessarily matter which individual genotype is reconstructed.

This assumption is invalid if recombination of individual genotypes generates new phenotypes and if the reconstructed ancestral gene itself is a consensus of those genotypes. Additional concerns arise if the reconstruction process generates inaccurate sequences, either because of bias in the evolutionary models used to infer ancestral states or because of phylogenetic conditions such as long branches and incorrect branching patterns[10–12].

Bias in the reconstruction process can, for example, lead to a preponderance of hydrophobic amino acids in an ancestral sequence. This bias results from long branches in a phylogeny combined with the fact that hydrophobic residues have high equilibrium frequencies in the amino-acid replacement matrices used to infer ancestral states. An increased proportion of hydrophobic residues in an ancestral protein has the potential effect of spuriously increasing thermostability. A similar form of bias is produced when the equilibrium frequencies themselves are incorrect. Equilibrium frequencies are derived from amino-acid occurrence in modern proteins. It may be incorrect to use these frequencies when reconstructing ancestral sequences if amino-acid occurrence has evolved over time, resulting in an inhomogeneous process[13].

We address these multiple concerns. First, we generate weighted random sequences sampled from the posterior distribution of ancestral character states to address whether bias in the amino-acid equilibrium frequencies affects the phenotypes of the inferred proteins. Second, we calculate ancestral amino-acid equilibrium frequencies and use these as an alternative to modern equilibrium frequencies to determine potential effects on ancestral phenotypes. Third, we reconstruct ancestral proteins across two competing bacterial phylogenies to determine the effects of topology on the ancestral phenotypes. For both phylogenies we assume the root of the tree to lie between bacteria and archaeans/eukaryotes, despite the suggestion that bacteria may be paraphyletic[14].

Elongation factor (EF) Tu (Bacteria)/1A (Archaea and Eukarya) is a suitable protein family with which to address the above concerns. The thermal stabilities of EFs are correlated with the growth temperature of their host organisms. Thus, EFs are optimally stable at temperatures of 20–45 °C, 45–80 °C and more than 80 °C when isolated from mesophiles, thermophiles and hyperthermophiles, respectively.

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This relationship is consistent with a correlation coefficient of 0.91 between the melting temperatures ($T_m$ values) of proteins and environmental temperatures of their host organisms[15].

Reconstructions of ancestral EF sequences were computed across two bacterial phylogenies selected from the literature[16,17]. Both phylogenies were constructed from the concatenation of numerous gene families and are therefore less susceptible to systematic error than phylogenies based on single genes. The two phylogenies capture the main competing views for bacterial relationships. One model posits that hyperthermophilic lineages occupy basal branches of the bacterial tree, whereas the other places these lineages in a more derived portion of the tree. To accommodate the latter model, a phylogeny was selected in which the Firmicute lineage (void of hyperthermophiles) was located at the base of the bacterial tree, although other topologies have been suggested[18].

The thermostability of modern and ancestral EF proteins was monitored by means of circular dichroism spectroscopy. The $T_m$ values of two modern EFs, from *Escherichia coli* and *Thermus thermophilus* (HB8), were determined as 42.8 °C and 76.7 °C, respectively. These values highlight the relationship between EF stability and the optimal growth temperature of their respective hosts, about 40 °C and about 74 °C (see Supplementary Information)[19].

$T_m$ values for ancestral EF proteins were determined across the two phylogenies (Fig. 1). The thermostability profiles of the ancestral proteins display the same general trend even though the two phylogenies represent competing hypotheses. Ancestral EF proteins resurrected at basal nodes are compatible with thermophilic environments, whereas ancestral proteins from more derived nodes are compatible with cooler environments. Consistent with this temperature trend is the observation that the node representing the presumed last common ancestor of bacteria (and thus the oldest) had the most thermostable protein within each phylogeny (64.8 °C and 73.3 °C). The similarity in thermostability (less than 9 °C difference) between these two ancestral proteins is significant because the sequences were identical across only 78% of the amino-acid sites (see Supplementary Information).

Systematic bias in the reconstruction of ancestral character states has the potential to generate incorrect inferences of ancient biomolecules[10]. As discussed above, we considered two potential sources of bias: first, incorrect equilibrium frequencies in the amino-acid replacement matrix, and second, selecting the M-PAS versus weighted random samplings from the posterior distribution of ancestral states. Ancestral amino-acid frequencies from a set of 31 protein families present in the last common ancestor of bacteria were

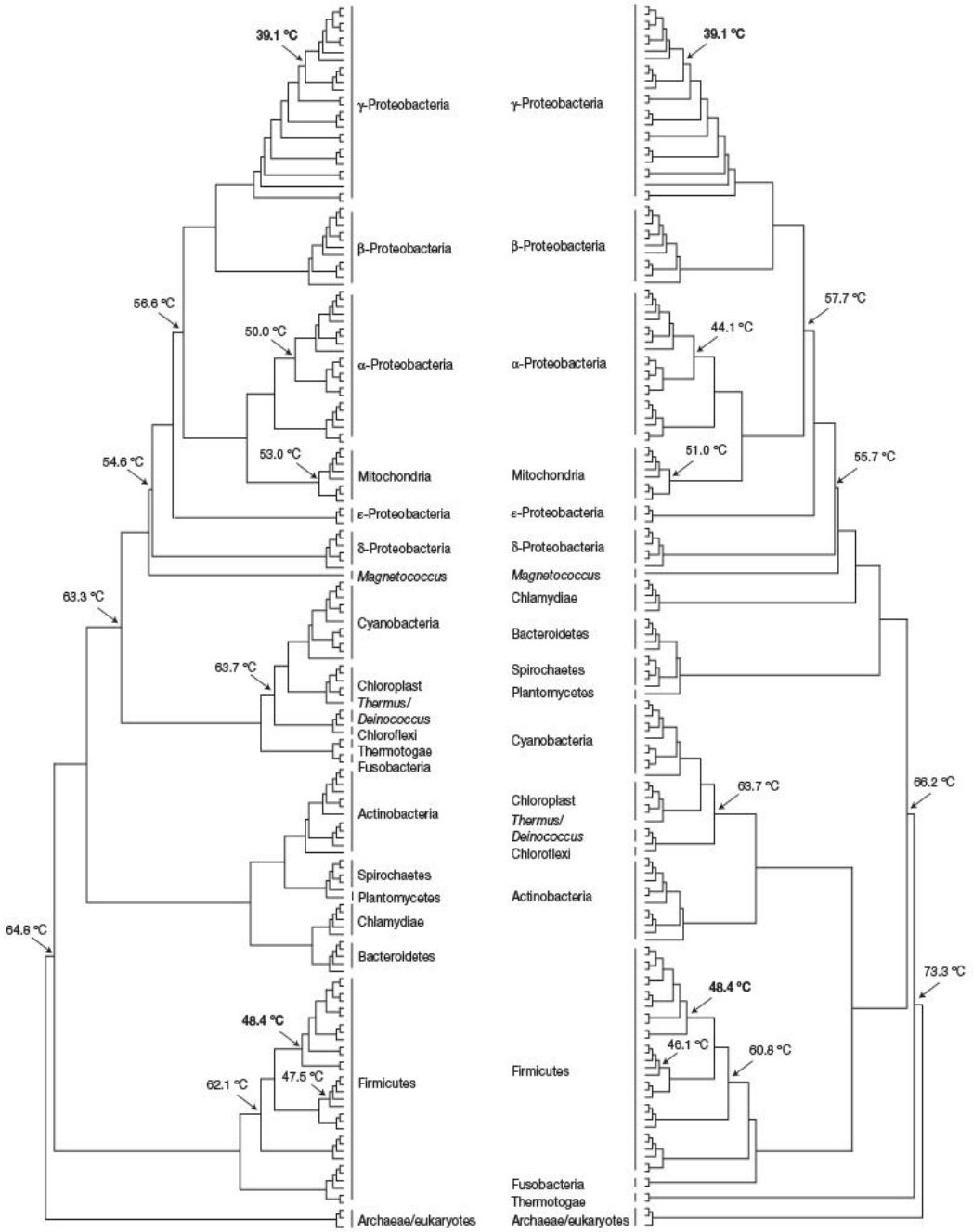


**Figure 1 | Cladograms of the trees used to resurrect EF proteins.** The phylogeny on the left follows ref. 17, whereas that on the right follows ref. 16. Melting temperatures for ancestral EF proteins are shown at their corresponding nodes. Temperatures in bold represent identical sequences at analogous nodes between the two trees. Errors associated with measurements of circular dichroism are negligible (see Supplementary Information).

estimated as described previously[13]. These 31 families were extracted from 16 species (8 mesophiles and 8 thermophiles) whose optimal growth temperatures ranged from 25 °C to 95 °C. The inferred ancestral frequencies then served as equilibrium frequencies in an amino-acid replacement matrix, and an ancient EF was reconstructed at the node representing the last common ancestor of bacteria from the phylogeny from ref. 17 by using this matrix. The ancient protein had a $T_m$ of 61.4 °C, in contrast with $T_m$ = 64.8 °C for the analogous ancient protein when standard frequencies were applied (Fig. 2). These two proteins differed at 14 amino-acid sites.

A random distribution of 10,000 sequences weighted in accordance with the posterior distribution for the node presenting the last common ancestor of bacteria from the phylogeny from ref. 17 was generated computationally to determine whether the EF data set and/or phylogenetic parameters led to bias when selecting the most probable ancestral character states. Five of the 10,000 sequences were then randomly selected and synthesized in the laboratory. The melting temperatures for these five sequences ranged from 60.0 °C to 66.3 °C, in contrast with $T_m$ = 64.8 °C for the M-PAS at this node (Fig. 2). The number of amino-acid differences between any of the five random sequences compared with the M-PAS ranged from 7 to 18 sites.

The environmental temperature of ancient bacteria inferred from resurrected EF proteins can be connected to divergence times of major bacterial lineages to gain a more detailed understanding of temperature trends for Precambrian life[16]. Divergence estimates from ref. 16 were applied to nodes in the current study. Figure 3 highlights the progressive cooling trend of ancient EF proteins from about 3.5 Gyr ago to 500 Myr ago. This temperature trend is strikingly similar to the temperature trend of the ancient ocean inferred from the deposition of oxygen and silicon isotopes[3–5].

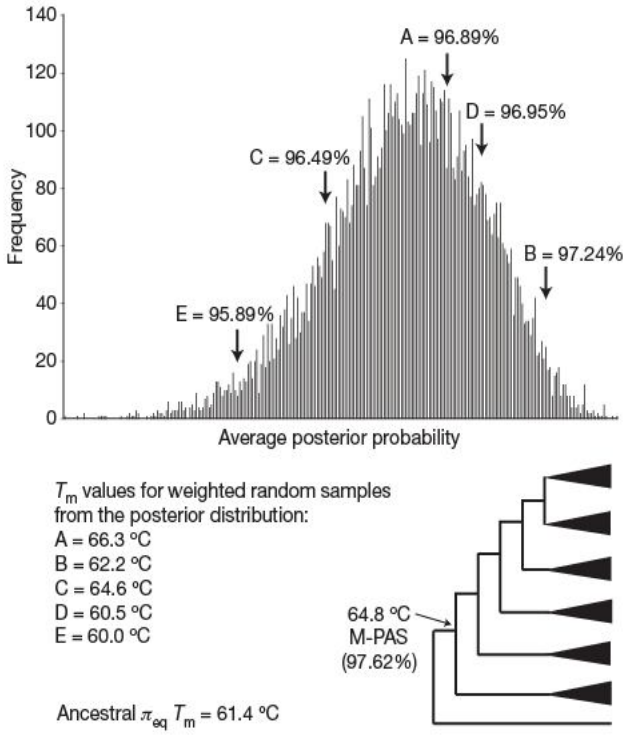


**Figure 2 | The EF phenotypes associated with samples drawn from the posterior distribution and ancestral equilibrium frequencies.** Both analyses focus on the node corresponding to the last common ancestor (LCA) of bacteria on the phylogeny from ref. 17. The figure shows the distribution of 10,000 weighted random sequences sampled from the posterior distribution associated with the LCA node. For each of the 10,000 sequences, the average posterior probability across all 394 sites is presented on the *x*-axis and the frequency of each average is presented on the *y*-axis. The distribution mean is 96.64%. Five sequences (A–E) were randomly selected from the distribution and synthesized. The corresponding $T_m$ values of the five encoded proteins are presented below the graph, and the average posterior probability across all sites for each sequence is indicated above an arrow. The $T_m$ for the LCA node inferred from ancestral equilibrium frequencies ($\pi_{eq}$) is also shown.

Reconstruction of ancestral EF proteins throughout the bacterial domain of life suggests that the organisms that hosted these extinct biomolecules lived in environments that have cooled progressively for about 3 Gyr. This evidence is predicated on multiple assumptions. For instance, it assumes that the reconstruction of ancestral sequences recapitulates ancient phenotypes and that phylogenies and divergence dates capture the evolutionary relationships and timing of bacterial divergences.

The observation that five samples from the posterior distribution had equivalent thermostability profiles to that of the M-PAS (Fig. 2) suggests that ancestral resurrections are robust for phenotype even when uncertainties exist in the ancestral sequences themselves. Further, the observation that ancestral amino-acid equilibrium frequencies produce an ancient protein with a phenotype equivalent to that of an ancient protein derived from modern amino-acid frequencies (Fig. 2) demonstrates that ancestral phenotypes can be robust to violations of a priori parameters contained within evolutionary models.

The inability (other than by time travel) to know the true relationships of bacterial lineages and their divergence times should not preclude attempts to understand Precambrian life. Rather, a coherent description of ancient life can be generated when empirical evidence from diverse studies converge on analogous conclusions. For instance, the same palaeotemperature trend was observed for ancestral EF proteins regardless of the phylogeny. For the phylogeny with divergence dates, this trend was substantiated when aligned with the inferred palaeotemperature curve of the ancient ocean.

These descriptions are particularly useful when they have predictive value. For instance, the last common ancestor of the mitochondrial bacterium is estimated to have lived 1.66–1.88 Gyr ago, on the basis of the $T_m$ values for ancestral EF proteins from the node representing the origins of mitochondria (51.0–53.0 °C) (see Supplementary Information). This is consistent with the origins of mitochondria estimated at 1.8 Gyr ago on the basis of a molecular clock[20], despite the controversial nature of the clock[21] and assuming that the last common mitochondrial bacterium lived at a time close to the endosymbiotic event between α-Proteobacteria and eukaryotic cells.

Our results suggest that early life lived at an environmental temperature similar to those of today's hot springs. Particular geological

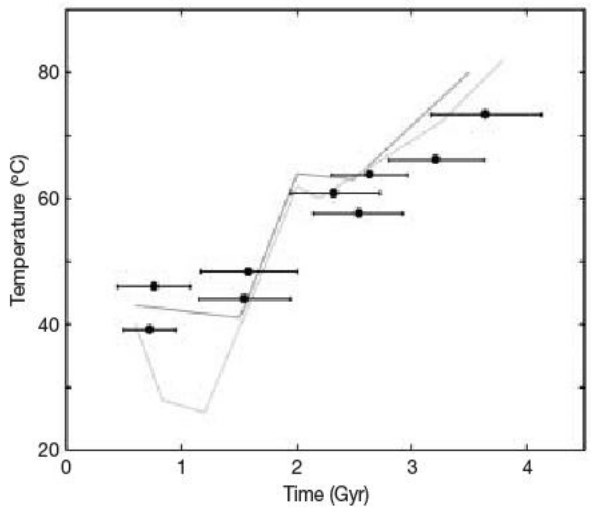


**Figure 3 | Plot of ancestral EF melting temperatures against geological time.** Molecular clock estimates are shown with their confidence intervals (horizontal bars) from ref. 16, using a 2.3-Ga minimum constraint for the Great Oxidation Event. Solid lines are temperature curves of the ancient ocean inferred from maximum $\delta^{18}O$ (light grey[3,4], dark grey[5]). Although not shown, an analogous trend is seen with $\delta^{30}Si$ isotopes[5].

theory and evidence suggest that the ancient ocean also had temperatures similar to those of hot springs[3,4,22]. As the ocean cooled from 3.5 to 0.5 Gyr ago, life may have responded by adapting its range of growth temperatures to correspond to its environment. This connection assumes that early life lived in the ancient ocean, which seems practical on the basis of geological and biological constraints such as ocean depth and circulation, land mass exposed to the atmosphere, and susceptibility to desiccation and ultraviolet radiation. Alternatively, it is possible that the inferred trend in palaeotemperature reflects an ecological trajectory as ancient bacteria made the transition from hot springs and thermal vents to the open ocean.

We note that correlating isotope ratios ($\delta^{18}O$ and $\delta^{30}Si$) with ancient ocean temperatures is contentious[23,24]. In particular, the correlation could be invalid if isotope ratios were caused by variation in seawater composition alone. This would translate into a more temperate ancient ocean and would be consistent with ancient glaciation events. However, the similarity in palaeotemperature trends inferred from $\delta^{18}O$, $\delta^{30}Si$ and ancient EF proteins is striking. Further, the overall trend is compatible with biological evolution. For instance, the thermostability of ancient EFs suggests that the origins of cyanobacteria occurred at an environmental temperature close to 63.7 °C (Fig. 1). This is consistent with an upper temperature limit of typical cyanobacterial mats in hot springs (about 65 °C)[25].

Overall, the results demonstrate that ancient EF thermostability profiles (phenotypes) are robust to uncertainties and potential biases associated with inferring ancestral character states (genotypes). The results also show how ancestral sequence reconstruction can connect the physical and natural sciences. As an extension, we have determined that certain ancestral EFs are indeed able to participate in peptide elongation when substituted for *E. coli* EFTu in a reconstituted *in vitro* translation system composed of *E. coli* components (data not shown). We expect that this type of assay will allow us to determine some of the underlying molecular mechanisms governing EF proteins as they evolved adaptively along particular branches during their evolutionary history.

## METHODS SUMMARY

Bacterial EF homologues were retrieved from GenBank, phylogenetic analysis was performed with MrBayes when necessary[26], and ancestral sequence reconstruction was calculated with PAML[27]. Ancestral amino-acid equilibrium frequencies were calculated as described previously[13].

Codon optimization and gene synthesis were conducted as described previously[28,29]. Ancestral and modern EF proteins were expressed by autoinduction and were purified by affinity chromatography[30]. Thermostability curves of EF proteins were determined by circular dichroism monitored at a wavelength of 222 nm. Data were analysed with MATLAB version 7.4.

**Full Methods** and any associated references are available in the online version of the paper at www.nature.com/nature.

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**Supplementary Information** is linked to the online version of the paper at www.nature.com/nature.

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**Author Contributions** E.A.G. designed the study, performed the evolutionary analyses and circular dichroism experiments, analysed the results and wrote the manuscript. S.G. performed gene synthesis. O.G. performed circular dichroism experiments, fitted the data and analysed the results. All authors discussed the results and commented on the manuscript.

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LETTERS

# Bacterial carbon processing by generalist species in the coastal ocean

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**The assimilation and mineralization of dissolved organic carbon (DOC) by marine bacterioplankton is a major process in the ocean carbon cycle[1]. However, little information exists on the specific metabolic functions of participating bacteria and on whether individual taxa specialize on particular components of the marine DOC pool[2]. Here we use experimental metagenomics to show that coastal communities are populated by taxa capable of metabolizing a wide variety of organic carbon compounds. Genomic DNA captured from bacterial community subsets metabolizing a single model component of the DOC pool (either dimethylsulphoniopropionate or vanillate) showed substantial overlap in gene composition as well as a diversity of carbon-processing capabilities beyond the selected phenotypes. Our direct measure of niche breadth for bacterial functional assemblages indicates that, in accordance with ecological theory, heterogeneity in the composition and supply of organic carbon to coastal oceans may favour generalist bacteria. In the important interplay between microbial community structure and biogeochemical cycling, coastal heterotrophic communities may be controlled less by transient changes in the carbon reservoir that they process and more by factors such as trophic interactions and physical conditions.**

The composition of marine bacterioplankton communities can readily be accessed through culture-independent analyses of 16S ribosomal RNA, yet there have been only limited opportunities to determine metabolic roles of the individual taxa[3]. For the marine carbon cycle, the complexity of the DOC pool and the taxonomic diversity of the heterotrophic bacteria that process it has impeded efforts to establish informative taxon–function linkages. Thus, the discrete roles of the heterotrophic marine bacterioplankton groups in the mineralization, export and storage of organic matter in the oceans are not understood and cannot serve as the basis for predictive models of carbon cycling in a changing ocean.

Dimethylsulphoniopropionate (DMSP) and vanillate are components of the DOC pool in coastal sea water. DMSP is released from marine phytoplankton into surface sea water, where it supports up to 10% of bacterial carbon demand[4]. Vanillate and other lignin monomers are released during the microbial processing of vascular plant detritus, and they contribute significantly to the DOC pool in marsh-influenced coastal waters[5]. A coastal bacterial assemblage (0.2–3.0 μm size fraction) was amended with 100 nM DMSP or vanillate in the presence of the thymidine analogue bromodeoxyuridine (BrdU)[6,7], and newly synthesized DNA was separated by immunocapture of BrdU-labelled DNA after 12 h. The captured DNA represented metagenomes of functional subsets of the bacterial community able to metabolize DMSP or vanillate. Bacterial assemblages without an added model DOC compound served as controls. Pyrosequencing produced 190,872 total reads from duplicate DMSP-specific metagenomes and 115,807 reads from duplicate vanillate-specific metagenomes (96 ± 16 bp (mean ± s.d.) per read; Table 1).

To confirm that the immunocapture protocol was effective at targeting newly synthesized DNA, we performed taxonomic fingerprinting by terminal restriction-fragment length polymorphism (T-RFLP). T-RFLP analysis showed that the 16S rRNA gene composition of immunocaptured DNA was distinct from that of the DNA that remained uncaptured (Supplementary Fig. 1). Further, when immunocapture was performed for samples without amendment with BrdU, no measurable DNA was recovered.

In the metagenomes, 16S rRNA genes accounted for 0.14% of the sequences (Table 1), in accordance with the expected frequency in genomes of cultured marine prokaryotes (0.19%; Supplementary Table 1). Comparisons between the DMSP-specific and vanillate-specific metagenomes for 16S rRNA sequences identified to the Order level showed similar taxonomic compositions (Fig. 1). Typical coastal ocean bacterioplankton taxa (ref. 8 and Supplementary Fig. 2) dominated both types of functional assemblage, including γ-Proteobacteria (61% in DMSP and 53% in vanillate; primarily Alteromonadales and Oceanospirillales), α-Proteobacteria (16% and 12%; primarily Roseobacter clade) and β-Proteobacteria (9% and 11%; primarily Burkholderiales). To a smaller extent, environmental clusters characteristic of oligotrophic oceans were present (SAR11, SAR116, SAR86, SAR92, SAR432, OM60/241 and OM185;

**Table 1 | Annotation statistics for experimental metagenomes**

| Parameter | *DMSP1* | *DMSP2* | *VAN1* | *VAN2* |
|---|---|---|---|---|
| Size of library (bp) | 9,481,910 | 8,696,512 | 3,900,971 | 7,137,286 |
| Number of sequences | 99,096 | 91,776 | 41,552 | 74,255 |
| Average sequence length (bp) (mean ± s.d.) | 96 ± 16 | 95 ± 17 | 94 ± 17 | 96 ± 16 |
| Number (%) of predicted 16S rRNA genes* | 134 (0.1) | 161 (0.2) | 53 (0.1) | 106 (0.1) |
| Number (%) of predicted functional genes* | 31,114 (31) | 23,860 (26) | 11,055 (27) | 26,259 (35) |
| Number (%) categorized by COG* | 15,864 (16) | 14,072 (15) | 8,347 (20) | 14,862 (20) |
| Number (%) categorized by KEGG pathway* | 6,652 (7) | 5,839 (6) | 4,182 (10) | 6,544 (9) |
| Number (%) categorized by SEED subsystem* | 12,598 (13) | 8,698 (9) | 3,520 (8) | 8,623 (12) |

* Cutoff values for BLAST were determined by *in silico* analysis of randomly fragmented known genes. An *E* value of less than $10^{-5}$, a hit length of more than 65 nt, and a similarity of more than 80% were used to predict 16S rRNA genes in BLASTN analysis against the Ribosomal Database Project. An *E* value of less than $10^{-2}$, a hit length of more than 23 amino-acid residues and a similarity of more than 40% were used to predict functional genes in BLASTX analysis against the NCBI nr database. An *E* value of less than $10^{-1}$, a hit length of more than 23 amino-acid residues and a similarity of more than 40% were used to predict functional genes in BLASTX analysis against the COG, KEGG and SEED databases.

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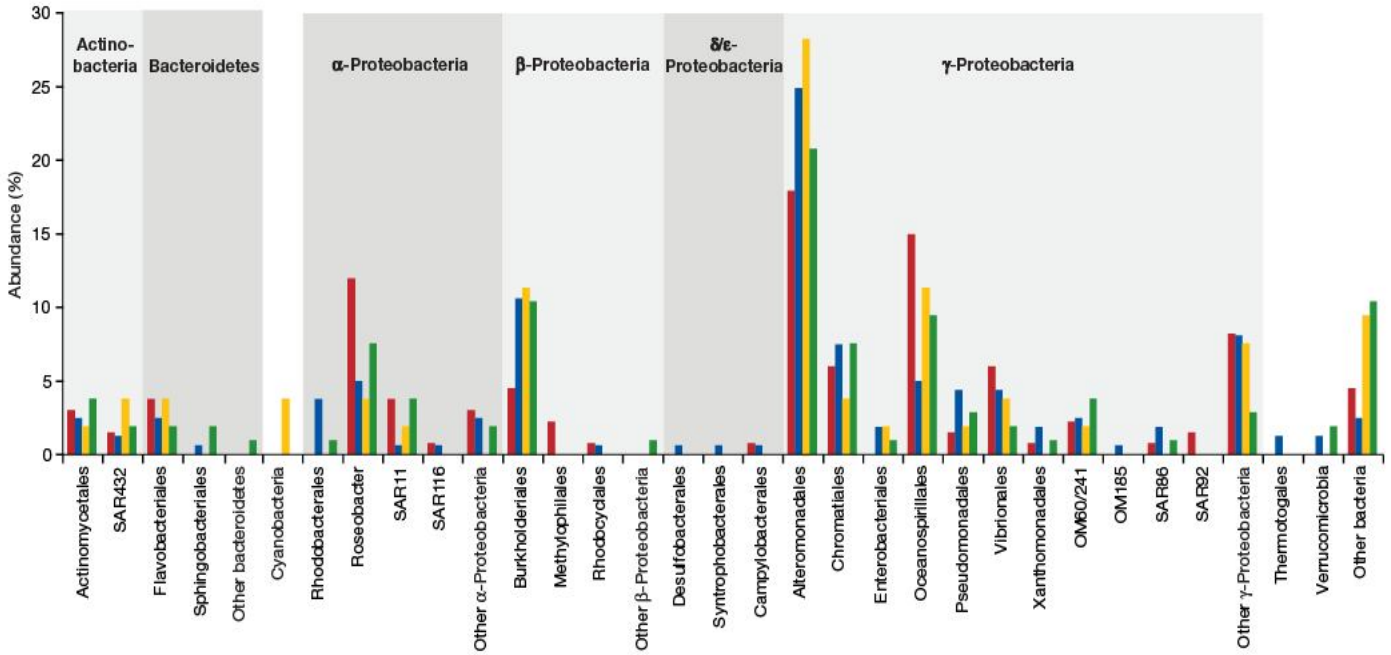


**Figure 1 | Apparent taxonomic distribution of 16S rRNA-like gene sequences recovered from metagenomes.** Red, *DMSP1*; blue, *DMSP2*; yellow, *VAN1*; green, *VAN2*.

1–4%), also with no apparent differences between metagenome types. Independent taxonomic analysis based on the amplification by PCR of 16S rRNA genes from the immunocaptured DNA confirmed the similar taxonomy of the DMSP-specific and vanillate-specific bacterioplankton assemblages, although the PCR-based methodology recovered fewer rare groups (Supplementary Fig. 3).

Similarity in 16S rRNA gene content of BrdU-labelled metagenomes might result if cell replication were to occur at the expense of organic matter pre-existing in the coastal sea water or released by experimental manipulations. However, cell numbers increased significantly for bacterioplankton communities amended with DMSP and vanillate (by 31% and 23%; two-tailed *t*-test, $P < 0.0001$ and $P = 0.0012$) but did not change in controls without carbon additions (Supplementary Fig. 4). Chemical analyses indicated that all added DMSP and vanillate was consumed within 12 h (Supplementary Fig. 5). Observed increases in cell numbers ($3.4 \times 10^5$ ml$^{-1}$) were comparable to expected increases if all growth was at the expense of DMSP or vanillate ($2.7 \times 10^5$ ml$^{-1}$ if cells contain 10 fg C and grow at 35% efficiency), indicating that most cell replication was supported by the added model DOC. 16S rRNA clone libraries from the initial and 12-h controls without carbon addition had comparable fine-scale taxonomic compositions (Supplementary Fig. 6).

For a direct comparison of metabolic abilities represented in the DMSP-specific and vanillate-specific assemblages, metagenomic sequences were annotated on the basis of homology to protein-encoding genes. *In silico* analysis of random fragments of known genes was first used to establish criteria for the gene predictions from the pyrosequences by BLAST analysis (Supplementary Figs 7 and 8); self-hits were excluded to mimic annotation of environmental sequences. With these criteria, 30% of the pyrosequences were identified as fragments of protein-encoding genes on the basis of sufficient homology to entries in the National Center for Biotechnology Information's non-redundant protein (NCBI nr) database (Table 1). The remaining sequences could not be definitively identified as genes encoding protein or rRNA, and probably represent unidentified genes, poorly conserved regions of known genes, or intergenic regions. The taxonomic bins of identified bacterial protein homologues were similar to those of the 16S rRNA sequences (Supplementary Fig. 9). On the basis of BLAST hits to essential single-copy genes[9], we estimate that 22 and 17 genome equivalents were represented by the protein-encoding genes in the two DMSP-specific metagenomes (*DMSP1* and *DMSP2*, respectively, and 9 and 17 genome equivalents in the vanillate-specific metagenomes (*VAN1* and *VAN2*, respectively) (Fig. 2). Because of the short length of the pyrosequences, only about one-tenth of each gene (96 bp average sequence length per 1,000 bp average bacterial gene length) was covered by the metagenomic sequence (that is, 0.1× coverage of each genome equivalent).

Two genes known to mediate the catabolism of DMSP were not overrepresented in the DMSP-specific metagenomes relative to the vanillate-specific metagenomes. Homologues to genes encoding the demethylation (*dmdA*)[10] and cleavage (*dddD*)[11] of DMSP were present in numbers sufficient to be found in about 18% and about 11% of the bacterial cells overall, but there was no pattern to their distribution (Table 2). Similarly, homologues to two genes encoding the catabolism of vanillate (*vanB* and *pcaH*) were present in about 17% and about 13% of cells, but with no bias towards the vanillate-specific

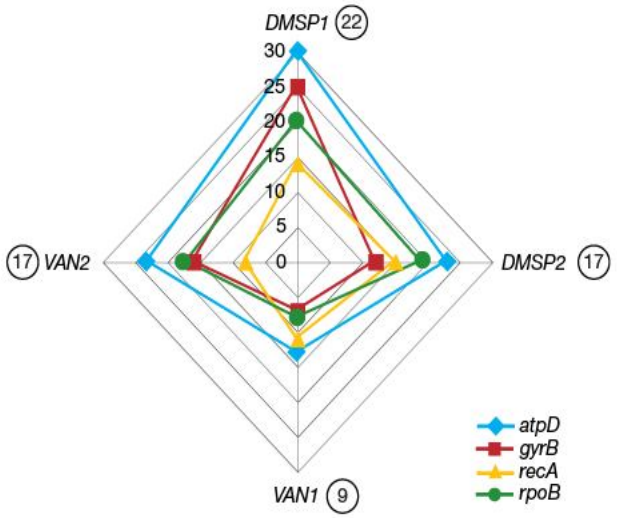


**Figure 2 | Estimated genome equivalents in experimental metagenomic data sets based on homologue counts for single-copy genes.** Raw counts were corrected for the effect of gene length on the probability of sampling by normalization to the length of *recA* (*recA*, 1,077 nt; *atpD*, 1,380 nt; *gyrB*, 2,418 nt; *rpoB*, 4,026 nt). The circled numbers show the estimated genome equivalent based on mean counts for the four single-copy genes. GenBank accession numbers for BLAST analysis: *atpD*, AAC76755; *gyrB*, ABG38537; *recA*, ABG32249; *rpoB* AAC76961.

**Table 2 | Estimated percentages of cells with selected carbon-cycle genes**

| Gene* | Function | Percentage of cells† | | | |
|---|---|---|---|---|---|
| | | *DMSP1* | *DMSP2* | *VAN1* | *VAN2* |
| *dmdA* | DMSP degradation | 4 | 17 | 32 | 18 |
| *dddD* | DMSP degradation | 10 | 12 | 19 | 3 |
| *vanB* | Vanillate degradation | 10 | 33 | 12 | 14 |
| *pcaH* | Protocatechuate degradation | 7 | 9 | 16 | 18 |
| *chi* | Chitin degradation | 4 | 10 | 0 | 0 |
| *CoxL*‡§ | Carbon monoxide oxidation | 50 | 73 | 44 | 46 |
| *PotA*§ | Polyamine transport | 40 | 23 | 22 | 41 |
| PR gene | Proteorhodopsin-based light harvesting | 39 | 25 | 16 | 0 |
| AAnP genes‖ | Aerobic anoxygenic phototrophy | 13 | 2 | 4 | 15 |
| *SoxB* | Inorganic sulphur oxidation | 20 | 18 | 7 | 23 |
| *Xsc* | Taurine degradation | 5 | 7 | 0 | 4 |
| *MtdB* | Methylotrophy | 0 | 10 | 0 | 0 |

* GenBank accession numbers of sequences used for BLAST analysis: AAV95190 (*dmdA*), EAV90715 and AAQ87407 (*dddD*), ABJ14290 (*vanB*), AAG03543 (*pcaH*), BAB21607 (*chi*), AAV94806 (*coxL*), AAV95654 (*coxL*), AAV94896 (*potA*), ZP_01054176, Q9F7P4 and EAQ40925 (proteorhodopsin), AAU00045 (*pufL*), ABN14037 (*pufM*), AAF24297 (*bchX*), AAV94301 (*soxB*), ABE35262 (*xsc*) and AAT02324 (*mtdB*).

† Raw homologue counts were corrected for differences in gene length by normalization to *recA* length as in Fig. 2. Corrected counts were converted to estimates of the percentage of cells containing homologues based on the equation percentage of cells = $100 \times H_{c,gene}/G_E$, where $H_{c,gene}$ is the length-corrected number of homologues of a given gene and $G_E$ is the estimated mean number of genome equivalents from Fig. 2.

‡ Sum of homologues from two clades of putative carbon monoxide dehydrogenases[29].

§ This may overestimate frequency because more than one copy of this gene has been found in genomes of some cultured marine bacteria.

‖ Average of homologue counts for three genes unique to aerobic anoxygenic phototrophy, namely *pufL*, *pufM* and *bchX* (ref. 30).

metagenomes. The relevance of these genes to DMSP and vanillate transformations by coastal marine bacteria has been demonstrated[10,12], although additional genes are likely to be involved[11].

Metagenomic sequences were assigned to COG (Clusters of Orthologous Groups) categories (2,620 categories), KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways (173 pathways) and SEED subsystems (519 subsystems) (Table 1). Statistical comparisons of protein predictions between DMSP-specific and vanillate-specific metagenomes by resampling[13] identified only 15 COG groups, 2 KEGG pathways and 10 SEED subsystems that were overrepresented in either the DMSP-specific or vanillate-specific metagenomes at a confidence level of 95% (Supplementary Table 2). These numbers are considerably lower than previous comparisons of metagenomic libraries with a comparable approach[14,15]. We conducted simulations with the COG data to estimate the disparity in assignments necessary to achieve statistical significance. A 1.5-fold difference in frequency could be detected if a category contained at least 13 sequences in the smaller library; greater fold differences were required for less abundant categories (Supplementary Fig. 10). Non-metric multidimensional scaling analysis of sequence assignments to COG categories indicated that the composition of metagenomes was no more similar within treatments than between them (Supplementary Fig. 11).

We searched the annotated genes for the presence of additional carbon-cycle-relevant capabilities, including carbon monoxide oxidation (*coxL*), chitin degradation (*chi*), methylotrophy (*mtdB*), polyamine transport (*potA*), taurine oxidation (*xsc*) and supplementary energy acquisition through proteorhodopsin and aerobic anoxygenic phototrophy (*pufL*). Despite incomplete coverage (Fig. 2), homologues to most genes were identified in both types of metagenome (Table 2), often in numbers at least equivalent to the DMSP and vanillate catabolic genes. Thus bacteria contributing genomes to the immunocaptured DNA had the ability to mediate various carbon transformations in addition to the specific phenotypes targeted.

The incubation time (12 h) relative to the expected generation times for coastal marine bacteria (4–84 h)[16] provided limited opportunity for major community shifts. It is therefore unlikely that rare bacterioplankton taxa overwhelmed the community during the time required for the accumulation of BrdU-labelled DNA. Further, most sequences from the PCR-based 16S rRNA clone libraries (about 95%) had best hits in BLASTN analysis to sequences obtained by culture-independent methods. Some immunocaptured DNA might originate from bacterioplankton using metabolites of DMSP and vanillate released by other taxa, and active cells incapable of assimilating BrdU would be missed by the capture protocol. However, neither of these caveats would affect our conclusion that the observed metagenome compositions are inconsistent with substantial metabolic specialization within the bacterioplankton community. These results do not exclude the possibility that specialists for DMSP and vanillate are present as minor members of this coastal bacterioplankton community that could dominate under conditions of constant supply[17], or that compounds less ubiquitous than DMSP and vanillate are metabolized by specialist taxa. Metagenomic sequencing of the control BrdU-labelled DNA might have shown more evidence for these groups. Nevertheless, the bacterial taxa poised to metabolize low-concentration pulses of common components of the organic matter pool in this coastal ocean are a taxonomically broad collection of metabolic generalists rather than a limited number of metabolic specialists.

Ecological theory predicts that heterogeneous environments favour the establishment of generalist species with broad ecological niches[18], although quantitative measurements of niche breadth have hitherto been extremely difficult to obtain[19]. Our direct sampling of the genetic capabilities of bacterioplankton performing defined roles in DOC processing (that is, the 'fundamental niche' concept applied to a bacterial assemblage)[20] by using an experimental metagenomics approach is in agreement with this theory, given the heterogeneity in supply rate and composition of organic matter to this coastal system[21]. It remains to be seen whether generalist bacteria are typical of marine environments with more predictable delivery of organic matter, such as the deep ocean or cold seeps. Bacterial generalists have also been proposed to dominate open ocean surface waters, although driven by the diverse pool of dilute substrates in oligotrophic sea water[22] rather than by heterogeneity in DOC supply. For ocean waters dominated by heterotrophic generalists, transient changes in the DOC pool may be less important than selective viral[23] or protistan[24] mortality or physical conditions[2,25] in determining short-term dynamics in taxonomic and genomic composition. Predictive modelling of biogeochemical processes in a changing coastal ocean requires a knowledge of how carbon-cycle functionalities are packaged into[26,27] and regulated within[28] individual bacterial cells.

## METHODS SUMMARY

**Metagenomic DNA.** Two sets of duplicate 20-litre microcosms were established with coastal sea water (collected in November 2005 at Sapelo Island, Georgia, geographical coordinates 81.2699° W, 31.3929° N) and amended with DMSP or vanillate (100 nM final concentration) and BrdU (10 μM). Two additional microcosms with BrdU only served as no-addition controls for manipulation effects. The BrdU-labelled DNA was extracted from microcosms after 12 h and was immunochemically purified with a modification of the method in ref. 6. Captured DNA from the treatment microcosms was sequenced by 454 Life Sciences.

**Pyrosequence annotation.** Unassembled pyrosequences were analysed by BLASTN against the Ribosomal Database Project II (RDPII) database to identify putative 16S rRNA sequences. Remaining sequences were analysed by BLASTX against the nr, COG, KEGG and SEED databases to identify putative protein-encoding sequences. *In silico* analyses of randomly fragmented nearly full-length 16S rRNA and protein-encoding genes from relevant bacterial taxa (Supplementary Table 3) were used to establish criteria for annotation (Supplementary Figs 7, 8 and 12).

**Analysis of PCR-based 16S rRNA sequences.** 16S rRNA gene sequences were amplified from initial, control and immunocaptured DNA by PCR. T-RFLP analysis was performed by digestion of carboxyfluorescein-labelled PCR amplicons with *Cfo*I (Roche). PCR amplicons were also cloned and sequenced from selected samples. Both T-RFLP profiles and 16S rRNA clone libraries showed no major composition shifts over 12 h in the absence of an added model compound (Supplementary Figs 6 and 13).

**Full Methods** and any associated references are available in the online version of the paper at www.nature.com/nature.

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**Supplementary Information** is linked to the online version of the paper at www.nature.com/nature.

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LETTERS

# Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands

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**Rates of atmospheric deposition of biologically active nitrogen (N) are two to seven times the pre-industrial rates in many developed nations because of combustion of fossil fuels and agricultural fertilization[1,2]. They are expected to increase similarly over the next 50 years in industrializing nations of Asia and South America[2]. Although the environmental impacts of high rates of nitrogen addition have been well studied[3–8], this is not so for the lower, chronic rates that characterize much of the globe. Here we present results of the first multi-decadal experiment to examine the impacts of chronic, experimental nitrogen addition as low as 10 kg N ha$^{-1}$ yr$^{-1}$ above ambient atmospheric nitrogen deposition (6 kg N ha$^{-1}$ yr$^{-1}$ at our site). This total input rate is comparable to terrestrial nitrogen deposition in many industrialized nations[2]. We found that this chronic low-level nitrogen addition rate reduced plant species numbers by 17% relative to controls receiving ambient N deposition. Moreover, species numbers were reduced more per unit of added nitrogen at lower addition rates, suggesting that chronic but low-level nitrogen deposition may have a greater impact on diversity than previously thought. A second experiment showed that a decade after cessation of nitrogen addition, relative plant species number, although not species abundances, had recovered, demonstrating that some effects of nitrogen addition are reversible.**

Biologically available nitrogen is the major limiting nutrient structuring most temperate terrestrial ecosystems, influencing ecosystem diversity, species composition and functioning[1,9,10]. Combustion of fossil fuels and modern agriculture have increased atmospheric nitrogen deposition from pre-industrial levels of approximately 1–3 kg ha$^{-1}$ yr$^{-1}$, to 7 kg ha$^{-1}$ yr$^{-1}$ over central and eastern USA, 17 kg ha$^{-1}$ yr$^{-1}$ over central Europe, and to as much as 100 kg ha$^{-1}$ yr$^{-1}$ over parts of the Netherlands[1,2,11,12]. Experiments in many ecosystems demonstrate that nitrogen addition at rates of 25 kg ha$^{-1}$ yr$^{-1}$ or more reduce plant species numbers and change ecosystem composition and functioning[3–8]. However, the short- and long-term ecological impacts of, and potential to recover from, the chronically elevated but lower rates of nitrogen deposition that characterize much of the Earth's land surface remain unclear.

Short-term mesocosm experiments[13], observational research along geographic nitrogen deposition gradients[14] and nutrient mass balance modelling[15] suggest that there may be ecosystem-specific rates of nitrogen deposition, termed $N_{crit}$ or critical nitrogen load, below which there are no negative ecological impacts[16]. Alternatively, effects of chronic nitrogen deposition at rates higher than pre-industrial levels may accumulate through time and eventually cause ecological impacts similar to those observed in shorter-term experiments that add nitrogen at higher rates[3–8]. For instance, recent observational studies of UK grasslands suggest plant species numbers may have decreased as a result of elevated nitrogen deposition[12,17], and show that species numbers correlate negatively with nitrogen deposition rates ranging from 5 to 35 kg N ha$^{-1}$ yr$^{-1}$, with no clear evidence of a threshold[12]. The few studies of the dynamics of plant diversity recovery after reductions of nitrogen input have had divergent results[18–21]; however, none have involved addition of nitrogen for as long and at such low rates as have our plots before cessation of treatment.

Here we use a 23-year field experiment to test these alternative hypotheses by determining the effects of chronic low-level rates of nitrogen addition, and its cessation, on numbers of grassland plant species. Our nitrogen-addition experiment was performed in two Minnesota prairie-like successional grasslands and in a native savanna grassland, each originally dominated by a species-rich mixture of native $C_4$ grasses and forbs[22] (Data access is available through the Cedar Creek Long Term Ecological Research Site website at http://www.cedarcreek.umn.edu/.) Plots received annual wet nitrogen deposition of approximately 6 kg ha$^{-1}$ yr$^{-1}$ (58% $NH_4$, 42% $NO_3$) and fertilizer nitrogen at 0, 10, 20, 34, 54 or 95 kg ha$^{-1}$ yr$^{-1}$ from 1982 to 2004. There were six replicates in each successional grassland and five replicates in the native savannah grassland (total 102 plots)[22]. To ensure primary limitation by nitrogen availability, all plots also received P, K, Ca, Mg and trace metals, none of which are limiting[22]. An additional site adjacent to one successional grassland was initially disk ploughed and had 36 plots receiving the same six nitrogen addition treatments as above ($n = 6$) for a decade (1982–1991). By that time, plant composition and diversity of the disk-ploughed nitrogen addition treatments had converged with those of the adjacent undisked treatments[23]. From 1991 onwards, all treatments were stopped for a randomly selected half of the six replicates per treatment in this additional site to observe recovery ($n = 3$). We measured plant species number and biomass in each plot every year from 1982 to 1994, and at least two of every three years from 1995 to 2004. Because climatic variation and other factors also affected plant species numbers[24], we calculated relative species numbers by dividing the number of plant species observed in a plot in a given year by the mean number of plant species observed in control plots for that year and field.

Chronic experimental nitrogen addition reduced relative plant species number compared with controls, even at the lowest treatment addition rate (10 kg N ha$^{-1}$ yr$^{-1}$). Plot averages for 2002–2004 showed that chronic nitrogen addition significantly ($P < 0.05$) reduced relative species number at all treatment rates, including a 17% loss at the lowest rate of nitrogen addition (Fig. 1a; we define nitrogen input as the sum of experimental nitrogen addition and regional wet nitrogen deposition). In contrast, analysis for 1983–1985 (second to fourth years of the experiment) showed lower overall loss rates and no significant plant species loss at our lowest treatment rate (Fig. 1b). This suggests that effects of low nitrogen addition rates

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take years to occur, and short-term studies may underestimate the effects of low-level but chronic nitrogen addition on the loss of plant species (compare Fig. 1b with Fig. 1a).

The fitted curve of Fig. 1a shows a greater proportional loss of species, per unit of nitrogen added, at lower rates of nitrogen addition. Specifically, proportional species loss in 2002–2004 was better fit by a logarithmic than by a linear function of the rate of nitrogen addition (differences in Akaike's information criterion, $\Delta AIC = 6.5$, Supplementary Information). The slope of the fitted curve is greater at lower nitrogen addition rates; thus, lower nitrogen addition rates cause a larger loss of relative plant species number per unit of nitrogen added. This nonlinear relation may result from greater efficiency of nitrogen capture, use and retention by the $C_4$-dominated, species-rich plant community present at lower rates of nitrogen addition, and greater leaching loss of nitrogen by the $C_3$-dominated, species-poor plant community that became dominant at higher rates of nitrogen addition[5]. In contrast, for 1983–1985, logarithmic and linear fits were indistinguishable ($\Delta AIC = 0.52$, Supplementary Information).

How well might loss of species at high rates of nitrogen addition predict actual long-term loss from chronic nitrogen addition at low rates? Consider the dashed line from the origin (zero proportional species loss in control plots) to the mean proportional species loss with 95 kg ha$^{-1}$ yr$^{-1}$ of nitrogen added (Fig. 1). For the long-term data (2002–2004), this line shows that the highest nitrogen addition rate underestimated the observed proportion of species lost at the lowest nitrogen addition rate by 60% (predicting 6.7% as opposed to 17% loss). This suggests that long-term studies using high rates of nitrogen addition may poorly predict, and even underestimate, the impact of chronic low rates of nitrogen deposition.

Repeated-measures multivariate analyses of variance (MANOVAs) over sequential three-year intervals (for example 1983–1985, 1985–1987, etc.) highlight the number of years of treatment, at each treatment rate, resulting in a loss of relative species numbers. There were significant ($P \leq 0.01$) and consistent reductions of relative species numbers after three, five, five, seven and nine years, respectively, for experimental addition of 95, 54, 34, 20 and 10 kg N ha$^{-1}$ yr$^{-1}$ above the ambient rate of nitrogen deposition, corresponding to total nitrogen inputs (experimental addition plus regional wet deposition) of 303, 300, 200, 182 and 144 kg ha$^{-1}$ (Fig. 2, and Supplementary Table 1). Thus, longer periods resulted in detection of loss of plant species relative to controls for progressively lower rates of nitrogen addition. Absolute species numbers in treated plots diverged from controls similarly to relative species numbers (Supplementary Fig. 1).

Because relative species number was reduced at all rates of nitrogen input, $N_{crit}$ must be lower than our lowest input rate. To estimate $N_{crit}$, we extrapolated our fitted curve and its confidence intervals (Fig. 1a), which predicted $N_{crit}$ as 5.3 kg N ha$^{-1}$ yr$^{-1}$ with a 95% inverse prediction interval of 1.3–9.8 kg N ha$^{-1}$ yr$^{-1}$. This estimate should be interpreted with care, as we lack data at sufficiently low input rates to estimate $N_{crit}$ definitively. However, our work demonstrates that $N_{crit}$ is below our lowest nitrogen input rate, and suggests that it may be lower than the 10–20 kg N ha$^{-1}$ yr$^{-1}$ previously estimated[25] for similar European ecosystems. Other approaches such as shelters to remove ambient deposition would refine this estimate of $N_{crit}$.

The decline in plant species number observed in 2002–2004 at our lower chronic rates of nitrogen addition (rates of 34 kg N ha$^{-1}$ yr$^{-1}$ and lower) was mainly from loss of rare species, where rare species are defined as those with a relative abundance of less than 1% in control plots on average across the entire experimental period (Supplementary Information). The number of rare species was reduced by nitrogen addition ($N_{add}$), differed among fields (Field)

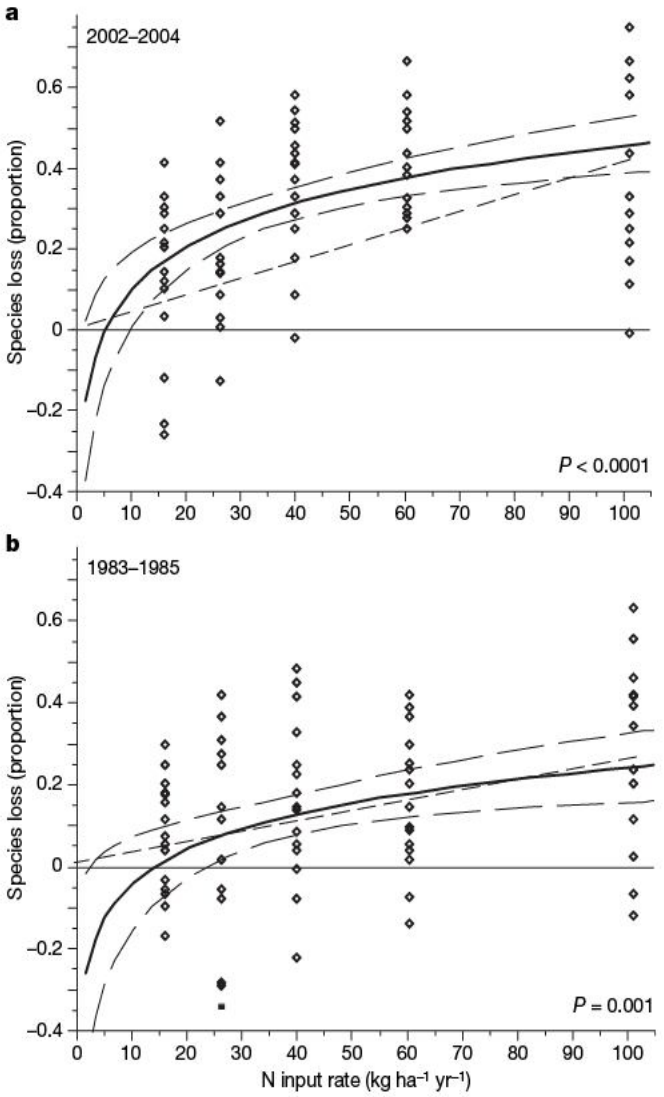


**Figure 1 | Proportional species loss versus nitrogen input rate for (a) 2002–2004 and (b) 1983–1985.** Shown are plot averages for each field over the three-year period fitted to a logarithmic curve excluding controls (95% confidence curves included). *P* values correspond to the significance of the nitrogen input term (N input = experimental N addition + atmospheric N deposition) in a model of the proportional loss of species regressed on the natural logarithm of the nitrogen input rate, Field, and their interaction (Supplementary Information). Dashed lines correspond to linear interpolation between the mean effect at the highest nitrogen addition rate and controls.

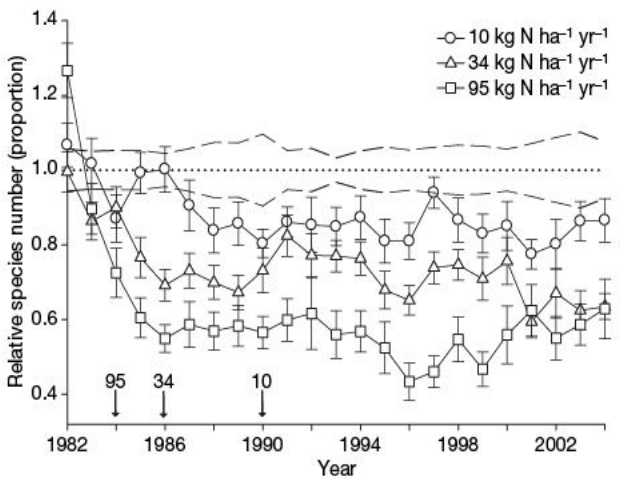


**Figure 2 | Relative species number versus time.** The treatment-specific average annual relative species numbers (± one s.e.m.) through time averaged over the three fields are shown. Dashed lines correspond to annual standard errors in control plots, and arrows indicate the year of first significant ($P < 0.01$) detection of relative species loss for a particular nitrogen addition treatment rate using MANOVA over three-year intervals (middle year highlighted). For clarity, only three of five nitrogen addition treatments are shown.

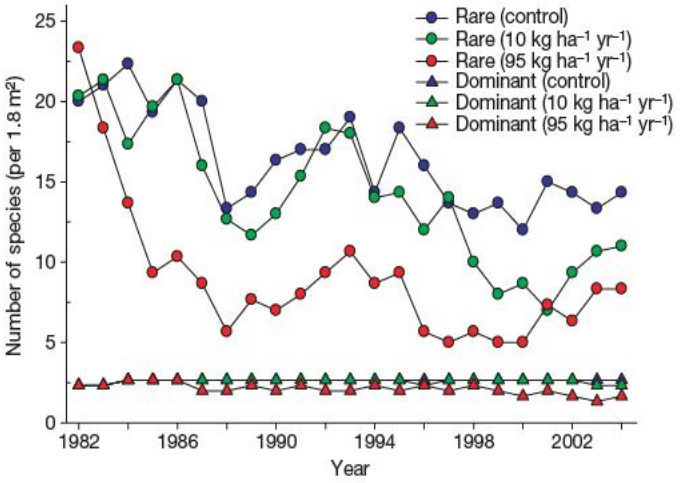


**Figure 3 | Losses of rare versus dominant species.** Dynamics of the numbers of rare and dominant species, expressed as the total numbers of such species across all replicates of a treatment in a field (see Methods). The average number across all fields of rare and dominant species in the controls (no added nitrogen) and in the lowest and the highest nitrogen addition treatments is shown. For clarity, intermediate nitrogen treatments and subordinate species are not shown, but demonstrated intermediate results.

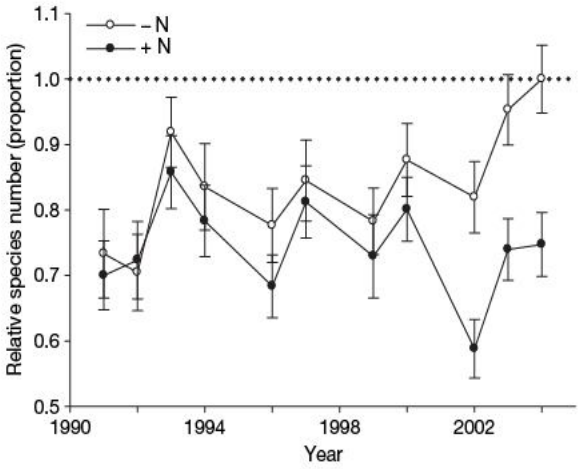


**Figure 4 | Recovery of relative species number after cessation of nitrogen addition.** Relative species number of all plots that continued to receive nitrogen (+N) and of those plots for which nitrogen addition ceased from 1991 and on (−N) is shown as the average across all nitrogen addition levels each year (± s.e.m.). There were no significant interactions between the rate of nitrogen addition and either year or the cessation treatment (Supplementary Information).

and decreased through time (Year) (Main effects model $F_{4,259} = 77.150$, $P < 0.0001$; $N_{add}$, $F_{1,259} = 105.090$, $P < 0.0001$; Year, $F_{1,259} = 180.712$, $P < 0.0001$; Field, $F_{2,259} = 11.398$, $P < 0.0001$; $P \leq 0.05$ for Tukey's honestly significant difference (HSD)-adjusted contrasts between controls and each nitrogen addition rate of 34 kg N ha$^{-1}$ yr$^{-1}$ and lower). Although all abundance classes were negatively impacted by nitrogen addition (Supplementary Information), there was a larger proportional and absolute decline in rare species numbers compared with other abundance classes (Fig. 3). For example, the average number of rare species (2002–2004) was lower at the treatment rate of 10 kg N ha$^{-1}$ yr$^{-1}$ compared with controls by 3.7 species or 26% (10.3 compared with 14 species), subordinate species by 1.4 species or 16% (7.8 compared with 9.2 species) and dominant species by 0.2 species or 8% (2.4 compared with 2.6 species). These trends in species losses were repeated at higher treatment rates (Supplementary Information). Logistic regressions of the presence or absence of individual species in each plot on the nitrogen addition rate (for rates of 34 kg N ha$^{-1}$ yr$^{-1}$ and lower) identified nine native perennial forb and grass species that were especially susceptible to loss after nitrogen addition (Supplementary Table 2: *Achillea millefolium*, *Asclepias tuberosa*, *Hieracium longipilum*, *Liatrus aspera*, *Panicum oligosanthese*, *Physalis virgiana*, *Schizachyrium scoparium*, *Solidago nemoralis* and *Viola pedatifida*).

In 1992 we ceased fertilizer treatment to observe recovery dynamics in an additional set of plots. Relative species numbers increased after cessation and converged with controls after 13 years (Fig. 4, and Supplementary Information). Rare species, disproportionately lost with nitrogen addition, recovered in relative numbers but not in absolute numbers over the 13 years of nitrogen cessation (Supplementary Information). In contrast, species composition showed few signs of recovery. Of the 15 most common species in the field from 1991 to 2004, comprising 95% of the total plant production over this period, only three responded to nitrogen cessation (Supplementary Table 3). *Schizachyrium scoparium* stopped declining and *Agropyron repens* stopped increasing in relative abundance, on average, in plots not receiving nitrogen. The sedge *Carex* was generally increasing with nitrogen cessation except at the highest prior rate of nitrogen addition (95 kg N ha$^{-1}$ yr$^{-1}$), where it was relatively unchanged (*Carex* individuals are not identified to species, but generally were *C. pensylvanica* or *C. muehlenbergii*[22]). Other studies of responses after nitrogen cessation have shown that plant populations[18–21] tend to recover more slowly than do plant tissue chemistry[21], soil pH[26] and nitrate leaching[21].

Much of the industrialized world currently receives nitrogen deposition at rates of 5–20 kg N ha$^{-1}$ yr$^{-1}$, with other regions expected to follow suit during industrial development over the next 50 years[2]. Our experiments demonstrate that grassland ecosystems on low-nitrogen soils were sensitive to chronic nitrogen inputs at low rates, and were capable of recovering some community properties within a decade after cessation of nitrogen addition. Because our plots are relatively small and frequently adjacent to plots of higher diversity, our observed rate of recovery is likely faster than would occur after reduction in regional nitrogen deposition. Determination of the generality of our results will require chronic low-level nitrogen addition experiments in various ecosystems. We suggest, however, that two aspects may prove general: first, that there are larger effects over the long term per unit of nitrogen if deposited at lower rates; and second, that many ecosystems that currently receive chronic nitrogen deposition at low rates, but elevated above pre-industrial levels, may be experiencing slow but chronic loss of biological diversity.

## METHODS

**Experimental design and data.** We only included the two later successional prairie-like grassland fields (fields B and C, abandoned from agriculture in 1957 and 1934, respectively) and the prairie opening in native savannah field (field D, never cultivated) in the analyses because the youngest field (field A, abandoned from agriculture in 1968) was relatively species poor and dominated by exotic invasive grasses at the beginning of the experiment. The initially disc-ploughed experiment was in field C only. Nitrogen was added as pelletized $NH_4NO_3$. Other treatments not included in this study include two higher nitrogen addition rates (170 and 270 kg ha$^{-1}$ yr$^{-1}$), which were excluded to restrict our comparisons to nitrogen deposition rates actually experienced globally, and one treatment with no nutrients added ('unamended'). We compare nitrogen addition plots with controls; however, similar results were obtained if we compared nitrogen addition plots with unamended plots. Because non-nitrogen nutrients were added and soil pH was controlled through addition of base cations, our study addresses the eutrophication effects of nitrogen addition while controlling for soil acidification, likely underestimating the total long-term impact of nitrogen deposition on species numbers[27]. We estimated the mean nitrogen deposition across the central and eastern USA by using all years of available data for sites from these regions in the EPA CASTNET program for total inorganic (wet plus dry) nitrogen deposition (http://www.epa.gov/castnet/). We determined the composition of local wet deposition from the on-site National Atmospheric Deposition Program monitoring station (Site MN01; http://nadp.sws.uiuc.edu/). Data for dry nitrogen deposition were unavailable at our site; however, examination of the three nearest sites in the EPA CASTNET program (sites PRK134, STK138 and VOY413) suggested that wet deposition is 72–84% of total nitrogen deposition, and is thus a reasonable proxy for total nitrogen deposition. See prior publications for additional information[5,22].

**Estimation of loss of relative species numbers for 2002–2004 and 1983–1985.** To determine the magnitude of relative species loss from each rate of nitrogen addition, we regressed the relative number of species on the rate of nitrogen input. To see if these relations changed through time, we conducted the same analyses early in the experiment (1983–1985) and two decades later (2002–2004). The proportional loss of species relative to controls was calculated as one minus the ratio of the plot average plant species number for the specified period (1983–1985 or 2002–2004) divided by the average number of species in the control treatment in that field over the same period. Analyses over the entire nitrogen addition gradient (0–95 kg ha$^{-1}$ yr$^{-1}$) required natural log transformation to meet assumptions of linearity. Control plots were excluded from the regressions to allow the $x$ intercept to vary more freely. We found an increase in proportional species loss with chronic nitrogen inputs for the 2002–2004 period (Fig. 1a; proportional species loss = $-0.26 + 0.16 \times$ log(nitrogen input rate); log(nitrogen input rate), $F_{1,83} = 25.204$, $P < 0.0001$). Plot averages over 1983–1985 show a less dramatic reduction (Fig. 1b; proportional species loss = $-0.33 + 0.12 \times$ log(nitrogen input rate); log(nitrogen input rate), $F_{1,83} = 10.549$, $P = 0.002$). Over either period, 'Field' was a significant predictor of species loss; and there were no higher-order interactions (Supplementary Information). Linear versus logarithmic models of the nitrogen input rate were compared using differences in Akaike's information criterion (ΔAIC).

**MANOVA of temporal trends.** To determine the amount of cumulative nitrogen added, at each rate of application, required to significantly reduce diversity, we used repeated measures MANOVA over three-year sequential periods beginning with 1983 (1983–1985, 1985–1987 and on to 2002–2004), comparing the average number of species between controls and each treatment level over the specified period. We began analyses with 1983 to correct for a one-year transient increase in diversity after nitrogen addition[21]. Inclusion of the first year of data did not qualitatively change the results. MANOVAs were run separately for each three-year period, and used the natural log of the nitrogen input rate and Field as independent variables (Supplementary Table 1). Annual tests for differences between controls and nitrogen treatments were also examined, though these were often non-significant at lower rates of nitrogen addition likely because of small differences at lower nitrogen addition rates and high annual variability[28]. In analyses using the entire 22-year dataset (1983–2004), all nitrogen addition levels had proportionally fewer relative numbers of species on average than controls at $P < 0.0001$. All within-subject tests (those including Time) for multivariate significance throughout this study are based on the Geisser-Greenhouse adjusted $F$-statistic. Between-subject tests (those not including Time) are based on exact $F$-tests.

**Loss risk for species at low nitrogen addition rates.** We used two methods to assess loss risk of species at low rates (rates of 34 kg N ha$^{-1}$ yr$^{-1}$ and lower) of nitrogen addition: (1) multiple regressions on the number of species in different abundance classes; and (2) logistic regressions on the presence or absence of individual species. Analyses over these low treatment rates did not require log transformations to meet model assumptions. For the first method, we classified each species as rare, subordinate or dominant based on an average relative abundance in control treatment of less than 1%, 1–10% and greater than 10%, respectively, over the entire experimental period (1982–2004), and then added up the number of unique species present across all replicate plots of a given treatment level in each field for each year. We then ran multiple regression analyses comparing the number of species in each abundance class with the nitrogen addition rate ($N_{add}$; rates of 34 kg N ha$^{-1}$ yr$^{-1}$ and lower), Field, Year (1983–2004) and all higher interactions (Supplementary Information). Poisson distributions were assumed for subordinate and dominant abundance classes owing to violation of normality assumption (Supplementary Information). For the second method, a species was counted as present if it was found in a plot for any one of the three years from 2002–2004, and absent only if not found for all three years (we tested for year effects and none were significant). Logistic regressions were run separately for species within fields. Tests for significance are based on likelihood ratio $\chi^2$ tests.

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LETTERS

# The coming acceleration of global population ageing

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**The future paths of population ageing result from specific combinations of declining fertility and increasing life expectancies in different parts of the world[1]. Here we measure the speed of population ageing by using conventional measures and new ones that take changes in longevity into account for the world as a whole and for 13 major regions. We report on future levels of indicators of ageing and the speed at which they change. We show how these depend on whether changes in life expectancy are taken into account. We also show that the speed of ageing is likely to increase over the coming decades and to decelerate in most regions by mid-century. All our measures indicate a continuous ageing of the world's population throughout the century. The median age of the world's population increases from 26.6 years in 2000 to 37.3 years in 2050 and then to 45.6 years in 2100, when it is not adjusted for longevity increase. When increases in life expectancy are taken into account[2,3], the adjusted median age rises from 26.6 in 2000 to 31.1 in 2050 and only to 32.9 in 2100, slightly less than what it was in the China region in 2005. There are large differences in the regional patterns of ageing. In North America, the median age adjusted for life expectancy change falls throughout almost the entire century, whereas the conventional median age increases significantly. Our assessment of trends in ageing is based on new probabilistic population forecasts. The probability that growth in the world's population will end during this century is 88%, somewhat higher than previously assessed[4]. After mid-century, lower rates of population growth are likely to coincide with slower rates of ageing.**

Conventional measures of ageing are based on chronological age. They assume that a 60-year-old person in 1900 was just as old as a 60-year-old person in 2000 because each has lived the same number of years. However, would we say that the two have aged at the same rate? After all, the 60-year-old in 2000 would, on average, have many more remaining years of life. Population ageing is not only about there being more old people (by today's definition of what is old): it is also about people living longer lives[5]. To capture this important impact of increasing life expectancy on our lives, and on the definitions of what is age and what is old, we introduce and quantify three new indicators of age that explicitly take changes in the remaining life expectancy into account. Although traditional age still greatly matters for institutional arrangements such as pension systems in most countries, the alternative measures tell us more about the changing human condition in which more people can plan for a longer and healthier life with consequences for their behaviour.

The conventional measures considered here are the proportion of the population aged 60+ (Prop. 60+), the median age of the population (MA) and its average age (Aver. Age). The alternative approach to measuring the proportion of elderly people in the population does not depend on a fixed age boundary but, rather, on a fixed remaining life expectancy. We define Prop. RLE 15− as the proportion of the population in age groups that have a remaining life expectancy of 15 years or less (see ref. 6 for the suggestion of a similar measure). If longevity increases, the minimum age of people included in Prop. RLE 15− increases. The adjusted version of the median age is called standardized or prospective median age (PMA)[2,3]. It is the age of a person in the year 2000 who has the same remaining life expectancy as a person at the median age in the year under consideration. The change in the prospective median age over some time period is roughly the change in the median age minus the change in life expectancy at the median age.

The adjusted version of the average age is the population average remaining years of life (PARYL). It is the weighted average of age-specific remaining life expectancies, where the weights are the proportions of the population at each age[7,8]. PARYL gives us the average remaining years of life of population members. Unlike the other measures, PARYL goes down as a population ages. We intuitively think of populations being younger when, on average, its members have more years left to live and PARYL is higher.

Figure 1 shows four of these measures of ageing as they evolve over time for the global population. All six measures are listed in Table 1 for selected regions and dates (information for all regions is given in Supplementary Table 2). All of them indicate that ageing will continue throughout the century. The two most rapidly increasing indicators, the proportion of the population 60+ years old and the median age of the population, are based on the traditional definition of age, hence suggesting the need for institutional adjustments to cope with these expected increases. The proportion of the global population 60+ years old increases from 10.0% in 2000 to 21.8% in 2050 and then to 32.2% in 2100. The three measures that are adjusted for longevity change show a slower pace of change.

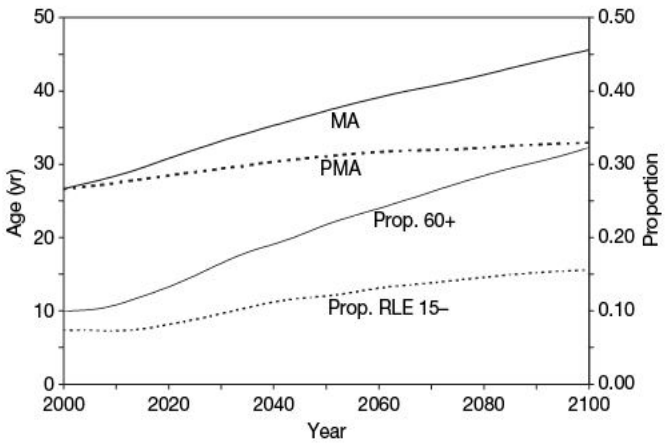


**Figure 1 | Projected changes in the level of ageing for the world population over the course of the century for four indicators of ageing as defined in the text.**

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**Table 1 | Indicators of ageing**

| Region | Indicator | 2000 | 2005 | 2010 | 2020 | 2030 | 2040 | 2050 | 2075 | 2100 |
|---|---|---|---|---|---|---|---|---|---|---|
| North America | Aver. Age | 36.5 | 37.0 | 37.7 | 39.5 | 41.3 | 42.6 | 43.6 | 46.5 | 49.5 |
| | Prop. 60+ | 0.16 | 0.17 | 0.18 | 0.23 | 0.27 | 0.28 | 0.30 | 0.35 | 0.39 |
| | PARYL | 43.0 | 43.3 | 43.4 | 43.5 | 43.6 | 44.1 | 45.0 | 46.3 | 48.4 |
| | MA | 35.9 | 36.7 | 37.2 | 38.4 | 40.3 | 41.9 | 43.0 | 47.0 | 50.0 |
| | Prop. RLE 15− | 0.11 | 0.10 | 0.10 | 0.11 | 0.13 | 0.15 | 0.14 | 0.15 | 0.15 |
| | PMA | 35.9 | 35.8 | 35.4 | 34.7 | 34.8 | 34.6 | 33.7 | 33.0 | 30.9 |
| Middle East | Aver. Age | 24.2 | 25.1 | 26.0 | 28.3 | 31.4 | 34.4 | 37.1 | 42.6 | 46.6 |
| | Prop. 60+ | 0.06 | 0.06 | 0.06 | 0.08 | 0.10 | 0.14 | 0.19 | 0.28 | 0.34 |
| | PARYL | 48.8 | 48.8 | 48.7 | 48.3 | 47.0 | 45.8 | 44.9 | 43.5 | 43.7 |
| | MA | 19.9 | 21.2 | 22.6 | 25.5 | 28.7 | 32.3 | 35.9 | 42.4 | 47.4 |
| | Prop. RLE 15− | 0.04 | 0.04 | 0.04 | 0.05 | 0.06 | 0.07 | 0.09 | 0.13 | 0.16 |
| | PMA | 19.9 | 20.3 | 20.9 | 22.0 | 23.5 | 25.5 | 27.6 | 30.0 | 30.6 |
| South Asia | Aver. Age | 26.5 | 27.1 | 27.8 | 29.8 | 32.2 | 34.6 | 37.0 | 42.4 | 47.3 |
| | Prop. 60+ | 0.07 | 0.07 | 0.08 | 0.09 | 0.12 | 0.14 | 0.17 | 0.26 | 0.35 |
| | PARYL | 44.1 | 44.1 | 43.9 | 43.2 | 42.1 | 41.2 | 40.4 | 38.6 | 37.6 |
| | MA | 22.7 | 23.4 | 24.5 | 26.9 | 29.6 | 32.8 | 35.9 | 42.6 | 48.5 |
| | Prop. RLE 15− | 0.06 | 0.06 | 0.06 | 0.07 | 0.08 | 0.10 | 0.11 | 0.16 | 0.19 |
| | PMA | 22.7 | 22.9 | 23.4 | 24.7 | 26.3 | 28.3 | 30.2 | 33.7 | 36.2 |
| China region | Aver. Age | 31.2 | 33.2 | 35.1 | 38.6 | 42.3 | 45.5 | 47.7 | 50.7 | 51.2 |
| | Prop. 60+ | 0.10 | 0.11 | 0.12 | 0.17 | 0.24 | 0.30 | 0.35 | 0.41 | 0.42 |
| | PARYL | 43.4 | 42.1 | 41.0 | 39.0 | 36.9 | 35.5 | 35.0 | 36.1 | 39.3 |
| | MA | 29.6 | 32.3 | 34.7 | 38.5 | 43.0 | 47.5 | 50.7 | 53.7 | 54.0 |
| | Prop. RLE 15− | 0.07 | 0.08 | 0.08 | 0.11 | 0.14 | 0.19 | 0.21 | 0.24 | 0.22 |
| | PMA | 29.6 | 31.7 | 33.5 | 36.0 | 39.3 | 42.3 | 44.1 | 43.0 | 38.6 |
| Pacific Asia | Aver. Age | 28.2 | 29.3 | 30.5 | 33.0 | 35.4 | 37.6 | 39.5 | 43.2 | 47.5 |
| | Prop. 60+ | 0.08 | 0.08 | 0.09 | 0.12 | 0.16 | 0.20 | 0.23 | 0.29 | 0.36 |
| | PARYL | 44.7 | 44.4 | 43.9 | 42.9 | 42.1 | 41.5 | 41.2 | 41.2 | 41.1 |
| | MA | 25.3 | 26.9 | 28.4 | 31.4 | 34.0 | 36.4 | 38.6 | 43.3 | 48.7 |
| | Prop. RLE 15− | 0.06 | 0.06 | 0.07 | 0.08 | 0.10 | 0.12 | 0.14 | 0.15 | 0.17 |
| | PMA | 25.3 | 26.2 | 27.1 | 28.7 | 29.9 | 30.9 | 31.6 | 32.4 | 33.7 |
| Japan/Oceania | Aver. Age | 40.4 | 41.6 | 43.0 | 45.7 | 47.9 | 49.7 | 51.3 | 54.1 | 57.7 |
| | Prop. 60+ | 0.22 | 0.24 | 0.27 | 0.31 | 0.35 | 0.40 | 0.42 | 0.47 | 0.51 |
| | PARYL | 41.3 | 41.0 | 40.6 | 39.7 | 39.5 | 39.5 | 39.6 | 41.1 | 43.0 |
| | MA | 40.0 | 41.3 | 42.8 | 46.7 | 49.9 | 52.1 | 53.9 | 57.6 | 61.1 |
| | Prop. RLE 15− | 0.13 | 0.13 | 0.14 | 0.17 | 0.18 | 0.18 | 0.20 | 0.21 | 0.21 |
| | PMA | 40.0 | 40.3 | 40.9 | 42.9 | 44.3 | 44.6 | 44.5 | 43.3 | 41.7 |
| Western Europe | Aver. Age | 38.3 | 39.1 | 40.1 | 42.4 | 44.7 | 46.8 | 48.4 | 51.0 | 53.5 |
| | Prop. 60+ | 0.20 | 0.20 | 0.21 | 0.25 | 0.31 | 0.34 | 0.37 | 0.42 | 0.46 |
| | PARYL | 41.0 | 41.0 | 40.8 | 40.3 | 39.8 | 39.6 | 39.7 | 41.4 | 43.5 |
| | MA | 36.8 | 38.3 | 40.0 | 43.1 | 45.8 | 48.2 | 50.2 | 53.5 | 56.5 |
| | Prop. RLE 15− | 0.13 | 0.13 | 0.13 | 0.14 | 0.15 | 0.18 | 0.19 | 0.20 | 0.19 |
| | PMA | 36.8 | 37.5 | 38.3 | 39.6 | 40.5 | 41.1 | 41.3 | 39.8 | 37.7 |
| Eastern Europe | Aver. Age | 37.0 | 38.4 | 39.8 | 42.7 | 45.6 | 48.2 | 50.3 | 52.4 | 52.4 |
| | Prop. 60+ | 0.18 | 0.18 | 0.20 | 0.25 | 0.29 | 0.36 | 0.42 | 0.44 | 0.44 |
| | PARYL | 39.7 | 39.1 | 38.5 | 37.3 | 36.0 | 35.3 | 34.9 | 36.9 | 40.6 |
| | MA | 35.6 | 37.1 | 38.9 | 42.9 | 47.3 | 51.3 | 54.0 | 55.7 | 55.7 |
| | Prop. RLE 15− | 0.13 | 0.13 | 0.13 | 0.15 | 0.18 | 0.19 | 0.22 | 0.24 | 0.21 |
| | PMA | 35.6 | 36.4 | 37.4 | 39.9 | 42.8 | 45.2 | 46.3 | 43.5 | 38.6 |
| World | Aver. Age | 29.7 | 30.4 | 31.3 | 33.1 | 35.2 | 37.1 | 38.8 | 42.3 | 45.5 |
| | Prop. 60+ | 0.10 | 0.10 | 0.11 | 0.13 | 0.17 | 0.19 | 0.22 | 0.27 | 0.32 |
| | PARYL | 43.8 | 43.6 | 43.3 | 42.8 | 42.1 | 41.6 | 41.3 | 41.0 | 41.2 |
| | MA | 26.6 | 27.5 | 28.4 | 30.8 | 33.2 | 35.3 | 37.3 | 41.4 | 45.6 |
| | Prop. RLE 15− | 0.07 | 0.07 | 0.07 | 0.08 | 0.10 | 0.11 | 0.12 | 0.14 | 0.16 |
| | PMA | 26.6 | 27.0 | 27.5 | 28.5 | 29.4 | 30.4 | 31.1 | 32.1 | 32.9 |

Prop. RLE 15− goes from 7.4% in 2000 to 12.0% in 2050, and then to 15.6% in 2100. As to regional differentials, Table 1 shows that Japan/Oceania is the oldest region today and is likely to keep this position throughout the century with its median age likely to increase to above 60 years. It is closely followed by the European regions. North America shows much slower ageing and is likely to be surpassed by China for every indicator of ageing by 2030–40.

Figure 2 shows the accelerating and then decelerating speed of ageing at the global level. It plots decadal changes in the level of the indicator divided by the maximum increase (speed) projected over the century. For all indicators, the speed accelerates over the coming years reaching the highest rate of increase before 2035. After that, the speed of ageing is expected to decelerate although there will be further increases in the level of ageing throughout the century. This analysis clearly shows that, even under widely differing definitions of ageing, the world is expected to experience a significant acceleration in the speed of population ageing over the coming years.

How certain are these projected future trends in ageing? Is the expected rapid increase in ageing in many parts of the world a near

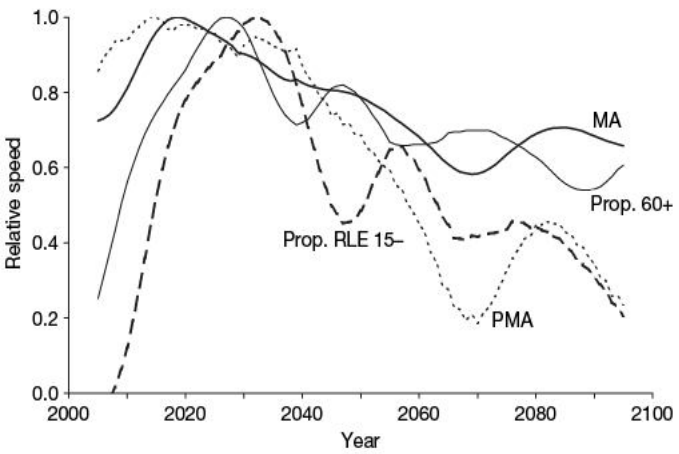


**Figure 2 | The changing speed of increase in selected indicators of ageing.** This is calculated as increases per decade in the level of the indicator divided by the maximum increase projected over the century; on the time axis, values are allocated to the middle of the decade considered.

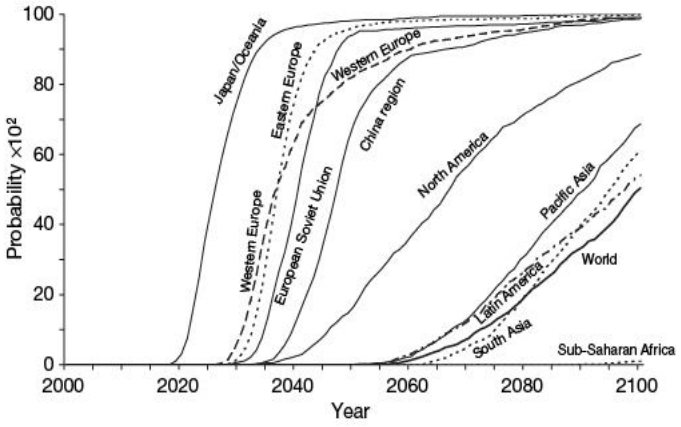


**Figure 3 | Cumulative probabilities of reaching a proportion 60+ of one-third or more for the world and selected world regions by calendar year.**

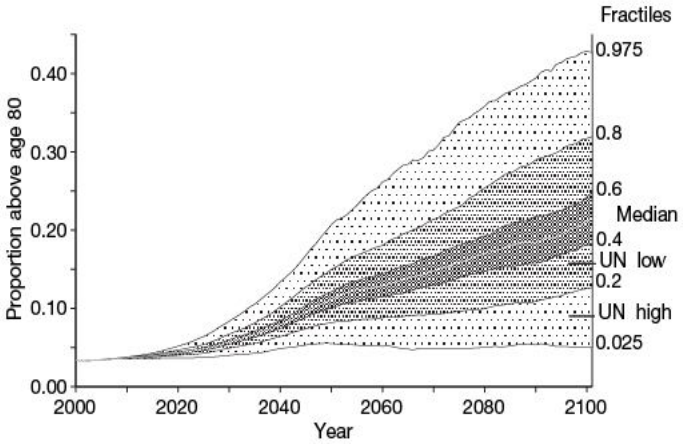


**Figure 4 | Fractiles of the projected uncertainty distribution of the proportion of the population above age 80 in Western Europe.** Straight lines in 2100 indicate the values given by the high and low variants of the United Nations (UN) long-term population projections.

certainty or just one out of several possible scenarios? The probabilistic nature of our population projections explicitly addresses this issue. Figure 3 shows the cumulative probabilities that different world regions reach one-third of their population 60+ years old (Prop. 60+) over the course of the century. By mid-century, the chance of having passed this specific ageing threshold is 98% in Japan/Oceania, 82% in Western Europe and even 69% in the China region. Uncertainty is so low in these regions because past fertility and mortality declines have already altered the age structures significantly. North America has a 50% chance of crossing this threshold in the 2060s owing to its currently still younger age structure and anticipated future migration gains. For sub-Saharan Africa, which still has an extremely young population with 44% of the population below age 15, the chance of Prop. 60+ being more than a third of the population is close to zero, even by the end of the century. For all other regions the chances start to increase over the 2060s and 2070s and reach around 50% by the end of the century. For the world as a whole, the cumulative probability turns out to be exactly 50% in 2100.

Figure 4 demonstrates another advantage of studying ageing from a probabilistic viewpoint. It shows predicted distributions of the proportion above age 80 for Western Europe (see Supplementary Table 1 for data on all regions). The proportion 80+ is almost certain to increase significantly over the coming decades. The projected increase in this indicator is very sensitive to the assumptions about future trends in old-age mortality where our assumed uncertainty ranges reflect tremendous disagreement among scientists[9–16]. Figure 4 shows that the 95% prediction interval is 5.5–20.7% by 2050 and 5.0–42.8% by 2100. The small lines inserted in 2100 give the results from the high and low variants of the most recent United Nations long-range projections[17]. These only reflect alternative fertility levels because the United Nations does not publish variants considering mortality uncertainty. That approach leads to a gross underestimation of the uncertainty of the future proportions of elderly.

Population ageing has many dimensions that will affect individuals and societies alike. When we supplement the conventional measures of ageing with ones that incorporate longevity change, we obtain a more complete understanding of how these dimensions are expected to evolve. In addition to changes in its level, the speed of ageing matters because, generally, the difficulties of adaptation to demographic change increase with the speed of change. In this respect, the world as a whole and the low fertility countries in particular face the challenge of an accelerating speed of ageing over the coming decades with the prospect of a slower speed of ageing at a higher level towards the second half of the century.

## METHODS SUMMARY

The population forecasts presented here are an update of earlier probabilistic forecasts published in 2001[4]. A fuller list of the results of this update is given in Supplementary Table 1. Although the methodology and the longer-term assumptions have not changed, the new forecasts reflect empirical trends and new data available up to 2006. One methodological innovation lies in the consideration of uncertainty ranges for starting conditions in certain regions of the world with unreliable information. This was particularly relevant for the assumed level of current fertility in China, where published total fertility rates range from 1.2 to 1.8. After a review of 18 different estimates[18], we assumed a median total fertility rate of 1.5 and an 80% uncertainty range from 1.3 to 1.7 as starting conditions. This change causes a downward shift in the projected long-term global population size, which is offset by the effects of the observed slower decline of fertility in sub-Saharan Africa, leaving forecasted world population sizes largely unaffected. Sensitivity analyses showed that the main findings about the coming acceleration of global ageing hold, even if China is excluded from the simulations. The proportion of our simulations that show a peak in the world's population some time during the century increases from 86% in our previous forecasts to 88% in our current ones (see Supplementary Figure 1). The forecasting methodology is described in ref. 4 and all the long-term assumptions are described and justified in detail in ref. 19. New short- and medium-term fertility assumptions are given in Supplementary Table 3. The mortality and migration assumptions were unchanged. The list of countries in each region appears in ref. 19.

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LETTERS

# Rapid appearance and local toxicity of amyloid-β plaques in a mouse model of Alzheimer's disease

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**Senile plaques accumulate over the course of decades in the brains of patients with Alzheimer's disease. A fundamental tenet of the amyloid hypothesis of Alzheimer's disease is that the deposition of amyloid-β precedes and induces the neuronal abnormalities that underlie dementia[1]. This idea has been challenged, however, by the suggestion that alterations in axonal trafficking and morphological abnormalities precede and lead to senile plaques[2]. The role of microglia in accelerating or retarding these processes has been uncertain. To investigate the temporal relation between plaque formation and the changes in local neuritic architecture, we used longitudinal *in vivo* multiphoton microscopy to sequentially image young APPswe/PS1d9xYFP (B6C3-YFP) transgenic mice[3]. Here we show that plaques form extraordinarily quickly, over 24 h. Within 1–2 days of a new plaque's appearance, microglia are activated and recruited to the site. Progressive neuritic changes ensue, leading to increasingly dysmorphic neurites over the next days to weeks. These data establish plaques as a critical mediator of neuritic pathology.**

To explore the formation of amyloid plaques and to determine the effects of newly formed dense-cored plaques on the microarchitecture of the brain, we have developed a novel *in vivo* multiphoton imaging technique. This recognizes newly formed plaques and allows monitoring of their immediate vicinity thereafter to determine the rate of their formation and the temporal sequence of pathophysiological events. We imaged young (5- to 6-month-old) B6C3-YFP mice, an age when plaques begin to appear[4] (Fig. 1). We used three-colour imaging to establish fiduciary markers for repeated imaging: YFP positive neurons, dendrites and axons in the cortex, methoxy-XO4-labelled fibrillar amyloid-β deposits in the parenchyma and on vessel walls, and a fluorescent angiogram with Texas red dextran to image blood vessels. A volume of cortex (lamina I–III) that initially did not contain plaques was re-imaged until repeat imaging detected a new plaque, establishing its 'birthday'. To ensure that the appearance of a new plaque did not simply reflect a greater depth of imaging or a slightly different imaging volume, we went through each image stack and compared them with previous sessions. New plaques were accepted only if a uniquely identifiable fiduciary point, such as a blood vessel or a dendritic process, could be unambiguously noted in a deeper imaging plane.

We postulated that we would occasionally observe the appearance and growth of new plaques within an imaging volume if the time interval between imaging sessions was long enough. From one weekly imaging session to the next, most of the sites remained unchanged (Supplementary Fig. 1a–c). However, we identified 14 new plaques: instances in which a plaque appeared in a second imaging session in a volume that had clearly been unoccupied in the first images one week earlier (Fig. 1a–c).

We examined the spatial relation between newly identified plaques and blood vessels. Measurements of the distance between vessel wall and the edge of a plaque confirmed that dense-core plaques develop close to but not within blood vessels (9.1 ± 3.9 μm from blood vessels). As a control, 70 randomly placed, plaque-sized objects had an average distance of 8.4 ± 11.2 μm from a vessel. New plaques therefore do not form any closer to vessels than would be expected by chance, in accord with an earlier study of human Alzheimer's disease[5]. Furthermore, multiphoton microscopic images showed that newly formed plaques were not penetrated by blood vessels[6], suggesting only a limited direct role of blood vessels in the formation of dense-core plaques.

To examine whether the phenotype of plaque formation in as short a period as one week was unique to the aggressive APP/PS1 transgenic mouse model, we used a mouse line that has a slower progression of disease (Tg2576)[7]. Seven Tg2576 transgenic mice (11 months) were imaged weekly, and fourteen additional new plaques were observed, suggesting that the rapid plaque formation is not restricted to one mouse model (Supplementary Fig. 2).

We next imaged the B6C3-YFP mice on a daily basis for up to six days in a row and/or on a weekly basis for up to three weeks (Fig. 1d–i). To our surprise, senile plaque formation is a very rapid event, with five new plaques appearing precipitously within 24 h after the last imaging session. However, plaque formation is a rare event. Combining our experiments of daily and weekly imaging, a total of 26 new plaques were found in 14 animals over 238 sites, imaged a total of 1,285 times.

These newly formed plaques were then re-imaged over days to weeks to determine their growth pattern. Measurements of plaque area over several imaging sessions revealed that they do not change in size after about the first 24 h, regardless of whether they had small or large diameters when they were first imaged (Fig. 1j). To examine whether imaging procedures had affected the observed plaque characteristics, we compared *in vivo* measures with those obtained from post-mortem analysis of mice either after our imaging protocols or with naive mice that had never been imaged. These analyses confirmed that the newly formed plaques are not significantly different in area or diameter from those observed post mortem and therefore represent typical plaques (Supplementary Fig. 3). Furthermore, the size distribution of new plaques compares well with that of all plaques seen post mortem in non-imaged mice (Supplementary Fig. 3). The shape of this histogram is also quite similar to that observed in analyses of human patients with Alzheimer's disease[8].

Several studies have shown that microglia surround senile plaques both in patients with Alzheimer's disease and transgenic mouse models[9,10] although their role remains elusive[11,12]. It has been proposed that activated microglia clear amyloid deposits[13], are the nidus for

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initiation of amyloid fibrils[14] or restrict plaques[15]. To study the interaction between newly formed amyloid plaques and microglia, we imaged microglia before and after plaque formation (Fig. 2) in PDAPP mice crossed with a line that expresses enhanced green fluorescent protein (EGFP) in microglia[16,17]. In this third APP transgenic line, we again found rapid formation of new plaques, with seven new plaques occurring within 24 h. Microglia were attracted to the site of plaque formation within a day (Fig. 2b). None of the new plaques occurred immediately adjacent to resident microglia, suggesting that microglia do not form the nidus of new plaques. We did not observe any plaques being cleared by this microglial response. This suggests that, unless further activated[18], microglia do not successfully clear plaques but instead may well restrict their growth, leading to the observed steady state of plaque size after initial formation.

Stokin *et al.* recently proposed that amyloid deposits followed axonal trafficking defects, marked morphologically as neuritic dystrophies. In contrast, amyloid deposition has been postulated to cause neuronal alterations[1,19]. To study the temporal relation between newly formed plaques and dystrophic neurites, we compared the shape and trajectories of yellow fluorescent protein

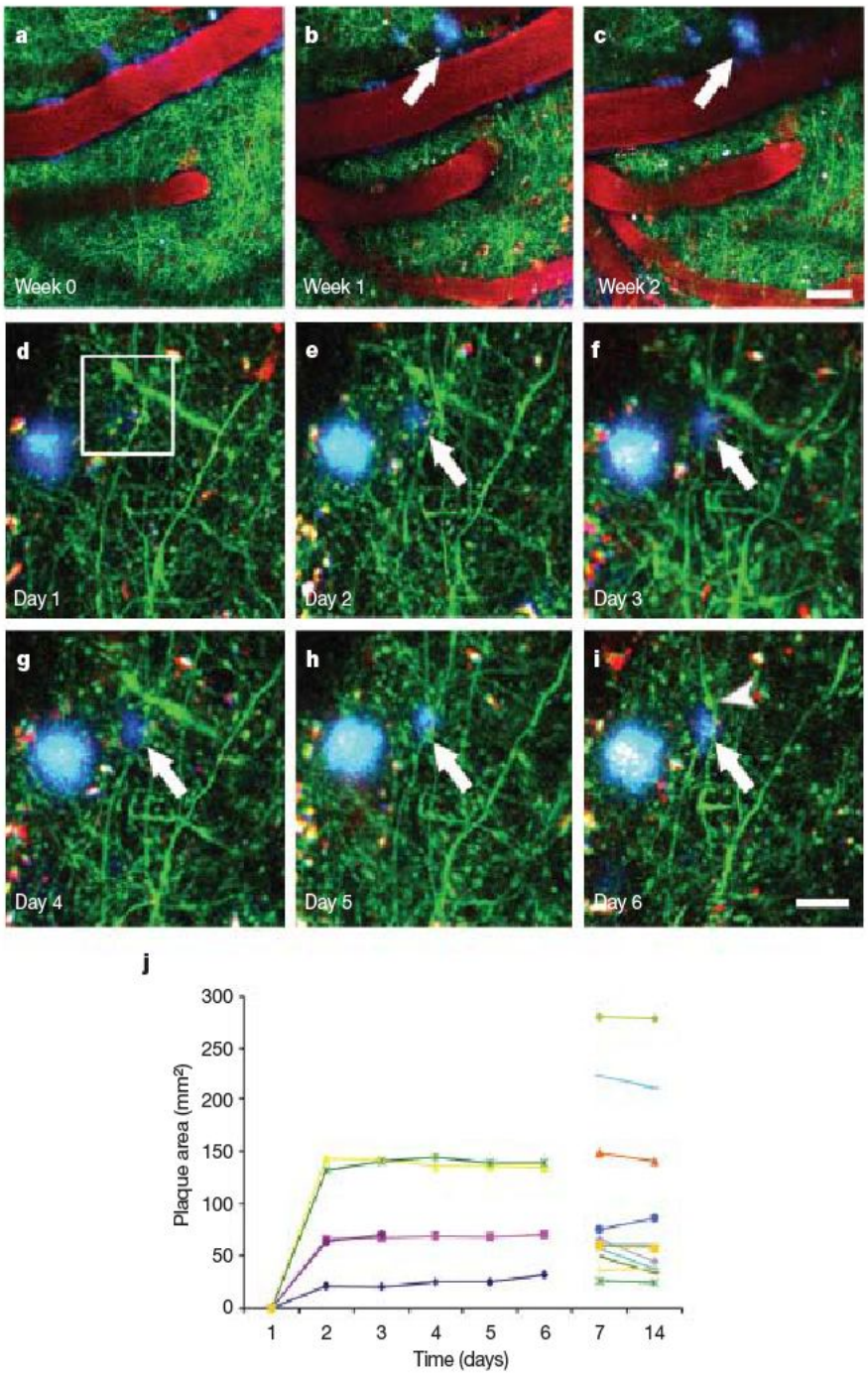


**Figure 1 | Appearance of a novel plaque is a rapid process.** **a–c**, Low-magnification images provide an overview of the areas of potential plaque formation. The angiogram (red, Texas red), amyloid deposition (blue, methoxy-XO4) and neurons (green, YFP) are easily identified on the initial day of surgery (**a**) as well as one week (**b**) and two weeks later (**c**), allowing re-imaging of the same sites over different imaging sessions. A new parenchymal amyloid deposition was identified one week (**b**) and two weeks (**c**) after the first imaging at this site. The new plaque appearing is indicated by an arrow (**b** and **c**). **d–i**, Sequential daily imaging of a potential plaque-formation area revealed that plaques can form rapidly in a short time interval from one day to another (**e**). The initial plaque was joined by a novel plaque marked with arrows over six consecutive days (**e–i**). Note the formation of a dystrophy indicated by an arrowhead (**i**). A line diagram was used in this figure to visualize plaque size over time. The mean area of new plaques was $88.7 \pm 69.3\ \mu m^2$ (mean ± s.d.) ($n = 18$). This is comparable to the mean plaque area of $93.7 \pm 74.8\ \mu m^2$ (mean ± s.d.) ($n = 153$) (Student's $t$-test not significant, $P = 0.77$) measured post mortem in a subset of these animals by using the Bioquant image analysis system. Each individual plaque that appeared either after one day or seven days is represented by a different colour (**j**). Scale bars, 30 μm (**a–c**) and 20 μm (**d–i**).

(YFP) fluorescent neurites before and after plaque formation in B6C3-YFP animals (Fig. 3). These neurites were morphologically normal in the volume of cortex one day before plaque formation. By contrast, in the days after plaque formation, progressive neuritic alterations were evident: from a smooth bend around the new plaque to very tortuous changes identical in appearance to dystrophic neurites seen in Alzheimer's disease[20]. The degree of neuritic deformation is best illustrated in individual image slices (Fig. 3a, c) as well as in three-dimensional reconstructions (Fig. 3b, d).

Neurite curvature was quantitatively analysed[20,21] from the *in vivo* images around each new plaque ($n = 12$) as well as more than 50 μm away from it, and compared with equivalent measures in randomly selected fields from YFP control mice. As expected, neurites were essentially straight, and unchanged in morphology over several weeks of imaging in YFP mice. In the B6C3-YFP mice, in the immediate vicinity of plaques the tortuosity increased gradually over the first week (Fig. 3e, $P < 0.0001$). Post-mortem analysis of neuritic curvature ($n = 218$) taken of randomly selected plaques observed at the time of death confirmed that neuritic curvature measured around new plaques one week after their formation is indistinguishable from the same measurements taken around all plaques ($1.048 \pm 0.02$ versus $1.052 \pm 0.014$). This suggests that neuritic changes occur rapidly and to essentially a maximal extent over the first week of a plaque's presence.

We next analysed changes in neurites near plaques on a daily basis. No change from baseline in neuritic curvature was detected on the day of plaque appearance (Fig. 3f). Neuritic alterations were first evident one day after the occurrence of a new plaque, indicating that neuritic deformation is a secondary effect of plaque development ($n = 15$; analysis of variance (ANOVA $P = 0.02$)). After two days the damage became more prominent; after five days it resembled the more robust neuritic damage seen after one week (Fig. 3e, f). Thus the neurite changes progressed over days after a short lag phase (although the imaging would not detect more subtle changes in cytoskeleton that might occur even earlier). In several instances, frank neuritic dystrophies appeared on a previously normal dendrite three to four days after the first appearance of a new senile plaque (Fig. 1). In almost all instances of new plaque formation (11 of 13 new plaques in which concurrent observation of YFP filled neurites was obtained), frank neuritic dystrophies followed plaque formation within one week (Supplementary Fig. 4a, b) ($\chi^2$ test $P < 0.001$). Post-mortem immunostaining suggested that both dendritic and axonal elements contribute to these dystrophic neurites (Supplementary Fig. 5).

Axonal dystrophies can be observed both near plaques and in the neuropil without a plaque. Such dystrophies have been hypothesized to anticipate the location of plaques[2]. We tested the idea that areas with a high density of dystrophies would be a prime location for plaque development. We tracked sites containing dystrophies but no plaques over days to weeks, but never observed a new plaque appearing at a site of high dystrophic neurite density. Instead, it seemed that these dystrophic processes are quite dynamic. Out of ten examples, 40% of the dystrophic neurites changed morphologies (with some even resolving completely) and only 60% were unaltered over one to two weeks of imaging (Supplementary Fig. 4c–f).

Because our *in vivo* amyloid imaging agent, methoxy-XO4, would not report amyloid-β deposits that do not contain β-pleated sheets, we compared immunostaining for amyloid-β and methoxy-XO4 in histologic specimens to look for diffuse plaques in 8-month-old B6C3-YFP mice. All of the plaques were dense-core in morphology, with co-staining of amyloid-β immunofluorescence and methoxy-XO4 (Supplementary Fig. 6). Because these mice develop innumerable plaques, the absence of any plaques with a diffuse morphology argues against the idea that dense-core plaques represent a remodelled form of diffuse, 'primitive' plaques.

Longitudinal *in vivo* imaging of plaque formation provides a new appreciation for the kinetics of the amyloid deposition process *in vivo*, and demonstrates the temporal relations between amyloid deposits, microglial recruitment and activation, and neuritic changes. Although it takes months for many plaques to accumulate, even in accelerated mouse models of Alzheimer's disease[7,16], our data show that an individual dense-core plaque's formation unexpectedly represents an acute event. These results are surprising because it has been generally accepted, based on *in vitro* studies of protein aggregation, that amyloid-β aggregation is time dependent and follows a relatively slow nucleation-dependent polymerization process[22]. It is possible that submicroscopic nuclei may be present in the cortex[23],

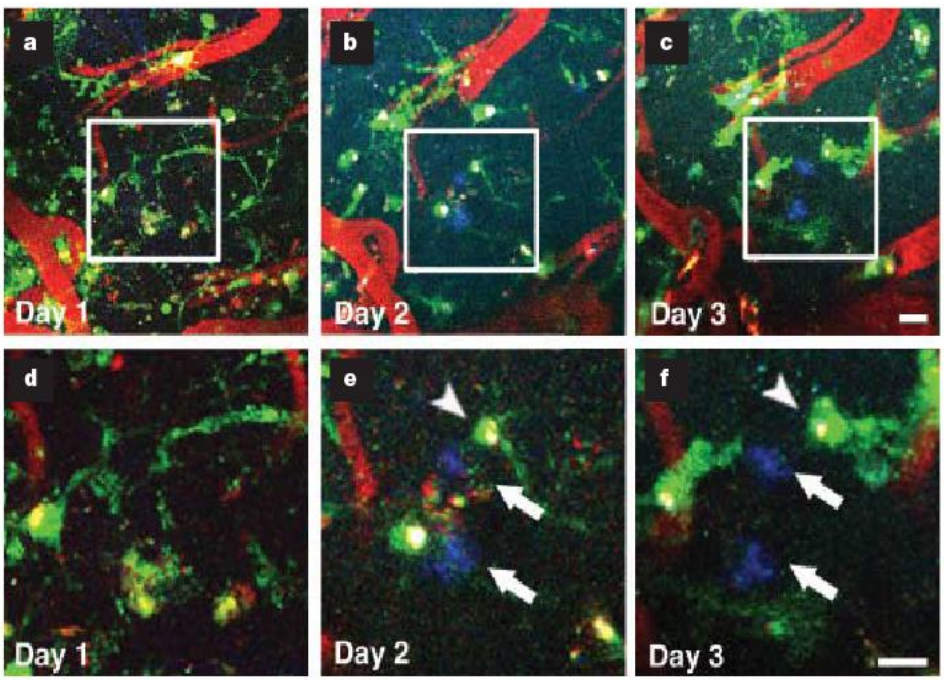


**Figure 2 | Microglia recruitment follows plaque formation.** **a–f**, Green fluorescent protein-positive microglia cells were imaged in PDAPP$^{+/-}$CX3CR1$^{+/-}$ mice before and after plaque formation in daily imaging sessions. **b**, **e**, Two plaques appeared within 24 h (arrows) as well as microglia around these newly formed plaques. Individual microglia remained stable but new microglia surrounding plaques were also evident (arrowhead). Scale bars, 20 μm.

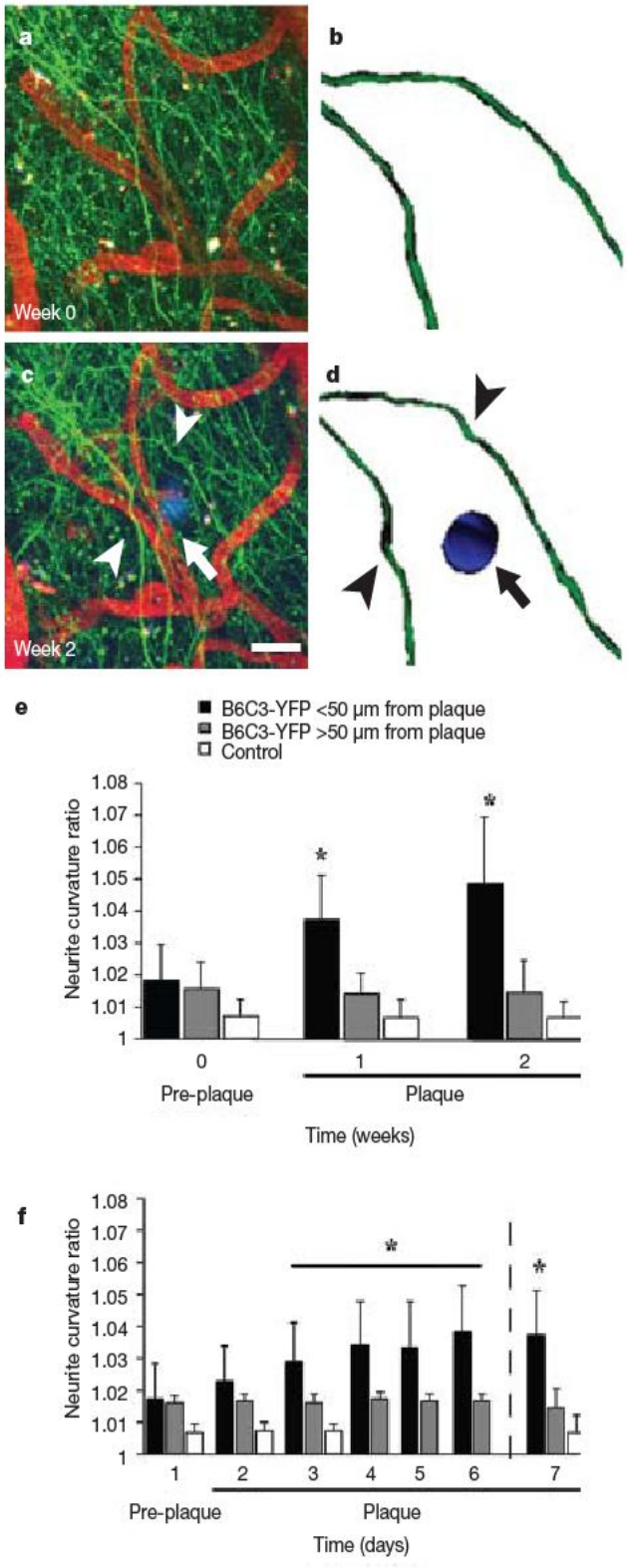


**Figure 3 | Plaque formation has no immediate effect on neuritic curvature.** **a–d**, Neurites were observed before and after plaque formation. A new plaque labelled with methoxy-XO4 is shown in **c** and indicated by an arrow. Images from the green channel were further analysed and neurite curvature was measured. Arrowheads in **c** and **d** denote the increased neurite curvature after a plaque formed. Three-dimensional reconstruction as an example of a developed plaque (blue) surrounded by deforming neurites (green) (**b**, **d**). Scale bar, 50 µm. **e**, Neurite curvature was measured one week before and after plaque development. Neurites ($n = 29$) measured in B6C3-YFP mice ($n = 6$) less than 50 µm from a plaque, neurites more than 50 µm from a plaque ($n = 27$) and neurites ($n = 40$) in control mice ($n = 5$) are depicted as black, grey and white bars, respectively. There was a statistically significant increase in neurite curvature after plaques form compared with the initial imaging session one week earlier. This increase persists one week after formation (two weeks from initial time point) (asterisk, ANOVA $P < 0.0001$). **f**, Daily assessment of neurite curvature changes ($n = 12$) from five new plaques revealed no significant differences at the day of plaque occurrence (data from all new plaques normalized to show plaque appearance at day 2). However, there was a tendency towards increased curvature, which became statistically significant at the third imaging day, one day after plaque appearance (asterisk, ANOVA $P = 0.002$). At imaging day 6, the neurite curvature ratio reached the highest level (asterisk, ANOVA $P = 0.0002$) and was comparable to that seen one week after plaque formation (day 7). Data are shown as mean ± standard deviation.

perhaps related to the recently reported amyloid-β oligomeric forms[24,25], as precursors to this sudden growth.

Observing the 'birthday' of new plaques provides the opportunity to examine directly whether dystrophic neurites near amyloid deposits precede or follow amyloid deposition. Our data strongly argue in favour of the latter, and suggest a period of several days after plaque formation during which progressive cytoskeletal derangements occur in neurites near a plaque. The observation of a local microenvironment in which microglia are recruited and activated after plaque formation further supports a model in which plaques act as a reservoir of bioactive molecules (Supplementary Fig. 7), which subsequently lead to neuronal alterations[19] including local loss of dendritic spines and axonal dystrophies[26]. These data thus lead to a model consistent with a prediction of the amyloid hypothesis in which amyloid deposition, activation and recruitment of microglia and local neuritic changes play out as a sequential cascade leading to neurodegeneration[1].

The current observations provide narrow parameters on the kinetics of plaque growth and stabilization through microglia, and raise the possibility that the years-long degenerative process of Alzheimer's disease is marked by innumerable sudden changes in cortical structure. Altering the kinetics of this process may well change the rate of progression of Alzheimer's disease.

## METHODS SUMMARY

Three different APP transgenic mouse lines were used in this study (B6C3-YFP, Tg2576 and PDAPP$^{+/-}$xCX3CR1-GFP$^{+/-}$). Cranial window surgery was performed followed by either daily or weekly *in vivo* multiphoton microscopy[26]. After completion of *in vivo* imaging, frozen brain sections were stained immunohistochemically. Image analysis measuring plaque size or neuritic curvature was done using Image J (National Institutes of Health freeware).

**Full Methods** and any associated references are available in the online version of the paper at www.nature.com/nature.

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**Supplementary Information** is linked to the online version of the paper at www.nature.com/nature.

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**Author Contributions** M.M.-L. and B.T.H. designed the study; M.M.-L., T.L.S.-J., C.P., M.G.-A., A.de C. and A.R. performed experiments; J.K.-T. and D.M.H. provided mice; M.M.-L. and B.T.H. wrote the manuscript; B.J.B. gave technical support and conceptual advice; B.T.H. supervised the study.

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LETTERS

# TANK-binding kinase-1 delineates innate and adaptive immune responses to DNA vaccines

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**Successful vaccines contain not only protective antigen(s) but also an adjuvant component that triggers innate immune activation and is necessary for their optimal immunogenicity[1,2]. In the case of DNA vaccines[3], this consists of plasmid DNA; however, the adjuvant element(s) as well as its intra- and inter-cellular innate immune signalling pathway(s) leading to the encoded antigen-specific T- and B-cell responses remain unclear. Here we demonstrate *in vivo* that TANK-binding kinase 1 (TBK1), a non-canonical IκB kinase, mediates the adjuvant effect of DNA vaccines and is essential for its immunogenicity in mice. Plasmid-DNA-activated, TBK1-dependent signalling and the resultant type-I interferon receptor-mediated signalling was required for induction of antigen-specific B and T cells, which occurred even in the absence of innate immune signalling through a well known CpG DNA sensor—Toll-like receptor 9 (TLR9) or Z-DNA binding protein 1 (ZBP1, also known as DAI, which was recently reported as a potential B-form DNA sensor[4]). Moreover, bone-marrow-transfer experiments revealed that TBK1-mediated signalling in haematopoietic cells was critical for the induction of antigen-specific B and $CD4^+$ T cells, whereas in non-haematopoietic cells TBK1 was required for $CD8^+$ T-cell induction. These data suggest that TBK1 is a key signalling molecule for DNA-vaccine-induced immunogenicity, by differentially controlling DNA-activated innate immune signalling through haematopoietic and non-haematopoietic cells.**

To develop optimal vaccines for clinical applications, it is important to understand the mechanisms of their actions on immune systems in terms of efficacy as well as safety. In particular, the innate immune recognition of the adjuvant element of vaccine formulations had been shown to be critical for its immunogenicity[2]. Many adjuvants, such as monophosphoryl lipid A and CpG DNA, seem to be ligands for TLRs[5,6]. In contrast, some conventional adjuvants, including aluminium hydroxide (alum) and incomplete Freund's adjuvant, as well as unconventional adjuvant-containing vehicles, such as apoptotic cells, are free of TLR ligand[7,8], suggesting that multiple innate immune recognition and signalling pathways are required for an adjuvant to function.

In the case of DNA vaccines, which have been shown to elicit humoral[9] and cellular[10] immune responses, unmethylated CpG motifs expressed within a plasmid backbone have been considered to be 'built-in' adjuvants, owing to their ability to activate the innate immune system by means of TLR9 (ref. 11). TLR9-deficient mice, however, mounted humoral and cellular immune responses to the encoded antigen comparable to those of wild-type mice[12,13]. Although another report showed a partial reduction of immune responses to a DNA vaccine in TLR9-deficient mice[14], the molecular and/or cellular mechanisms underlying the adjuvant effect and element(s) of DNA vaccines have not been fully clarified[15]. To address this issue, we used an optimized immunization protocol for DNA vaccination by electroporation as described previously[16,17]. After DNA vaccination, mice lacking TLR9 or its essential adaptor, MyD88, mounted both humoral and cellular immune responses to DNA vaccines comparable to those of wild-type mice (Supplementary Figs 1a–d and 2). Moreover, plasmid DNA electroporation activated dendritic cells to produce type-I interferons (IFNs) and inflammatory cytokines in a TLR9-independent manner (Supplementary Fig. 1e and data not shown). Although the immunogenicity of DNA vaccines may vary due to many factors such as the quality of plasmid DNA, injected sites, injection methods or modification of CpG motifs within plasmid DNA[11], our results support previous findings[12,13] indicating that TLR9-mediated recognition of plasmid DNA and subsequent signalling are not essential for optimal DNA vaccination.

Recent accumulating evidence, in contrast, suggests that the double-stranded structure of DNA, independently of CpG motifs, possesses immunomodulatory effects when introduced into the cytosol or its homeostatic clearance is hampered[18]. Intracellular administration of double-stranded B-form DNA (B-DNA) triggers TLR-independent, TBK1- and interferon regulatory factor 3 (IRF3)-dependent innate activation of both immune and non-immune cells to produce type-I IFNs and their inducible genes[19–21]. On the basis of these results, we hypothesized that the immunogenicity of DNA vaccines may be controlled by these TLR9-independent immunostimulatory activities of B-DNA as 'built-in' adjuvant(s), thereby prompting us to investigate whether TBK1-mediated innate immune activation contributes to DNA vaccine immunogenicity.

Because induction of type-I IFNs is a hallmark of TLR9-independent innate immune activation by B-DNA[19–21], which has been shown to have an important role in the following adaptive immune responses[22,23], we initially examined the effect of type-1 IFNs on the immunogenicity of DNA vaccines. IFN-αβR-deficient mice that lack type-I IFN-mediated signalling (*Ifnar2*$^{-/-}$) and wild-type mice were immunized with plasmid DNA encoding LacZ or the influenza NP protein. After immunization, wild-type but not *Ifnar2*$^{-/-}$ mice elicited strong T- and B-cell responses to LacZ, including serum LacZ-specific immunoglobulin (Ig)Gs (Fig. 1a, b), spleen $CD8^+$ T-cell frequency and IFN-γ secretion (Fig. 1c, d). Similarly, NP-specific IFN-γ production by $CD4^+$ or

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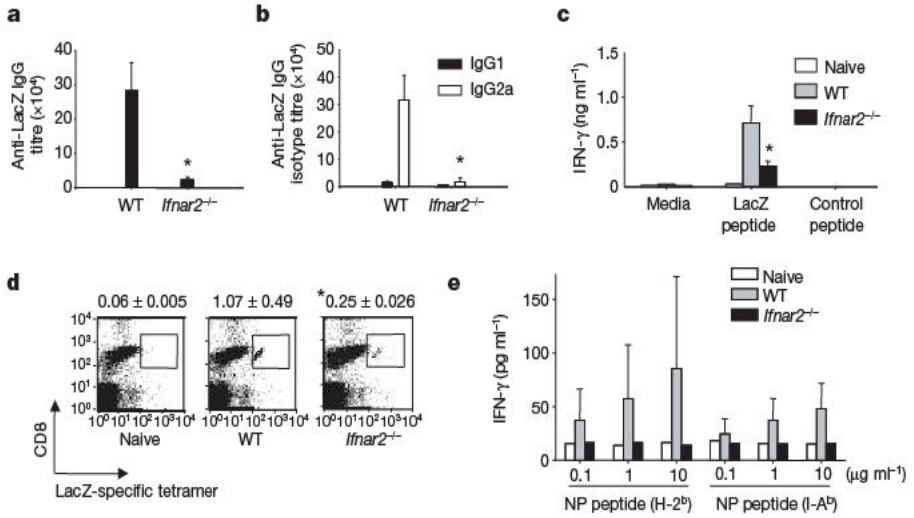


**Figure 1 | Optimal DNA vaccine immunogenicity requires type-I interferons.** Mice lacking type-I IFN signalling (*Ifnar2*$^{-/-}$) or wild-type (WT) mice were immunized twice by i.m. electroporation with a DNA vaccine encoding LacZ (**a**–**d**) or influenza A virus NP (**e**) protein at intervals of 4 weeks. The serum titres of anti-LacZ IgG (**a**), of IgG1 or IgG2a (**b**), and of spleen LacZ-antigen-specific IFN-γ production (**c**), as well as the frequency of CD8$^+$ T cells (**d**; average % of the gated population ± s.d.), were measured two weeks after the second immunization. Similarly, NP-specific IFN-γ production in the immunized spleen in response to NP peptides (I-A$^b$ or H-2$^b$) for CD4$^+$ or CD8$^+$ T cells, respectively, was also measured (**e**). Data are the averages ± s.d. of 3–5 mice per group; *$P < 0.01$ against wild-type mice.

CD8$^+$ T-cell-specific peptide was dependent on IFN-αβR (Fig. 1e). These results indicated that signalling by type-I IFNs is indispensable among the critical factors for DNA-vaccine-induced immunogenicity, although minimal immune responses were still observed in *Ifnar2*$^{-/-}$ mice.

We confirmed *in vitro* that plasmid DNA electroporation activated bone-marrow-derived dendritic cells to produce type-I IFNs in a TBK1-dependent manner (Supplementary Fig. 3a, b and data not shown), consistent with our previous findings with cationic liposomes[20]. To examine the role of TBK1 in DNA vaccination *in vivo*, we used TBK1-deficient mice on a *Tnf*$^{-/-}$ background[24]. Although *Tbk1*$^{-/-}$ mice die *in utero*, this lethal effect can be resuscitated in the absence of tumour necrosis factor (TNF)[24]. An advantage of using these mice is that deficiency of TBK1 or of TNF does not influence TLR9-dependent innate immune activation and vice versa[20]. Wild-type and *Tnf*$^{-/-}$ mice elicited comparable antigen-specific immune responses to the encoded antigen after DNA vaccination (Figs 2a and 3a, and data not shown). In contrast, however, *Tbk1*$^{-/-}$ mice on a *Tnf*$^{-/-}$ background failed to increase the frequency and cytotoxicity of antigen-specific CD8$^+$ T cells and spleen IFN-γ production

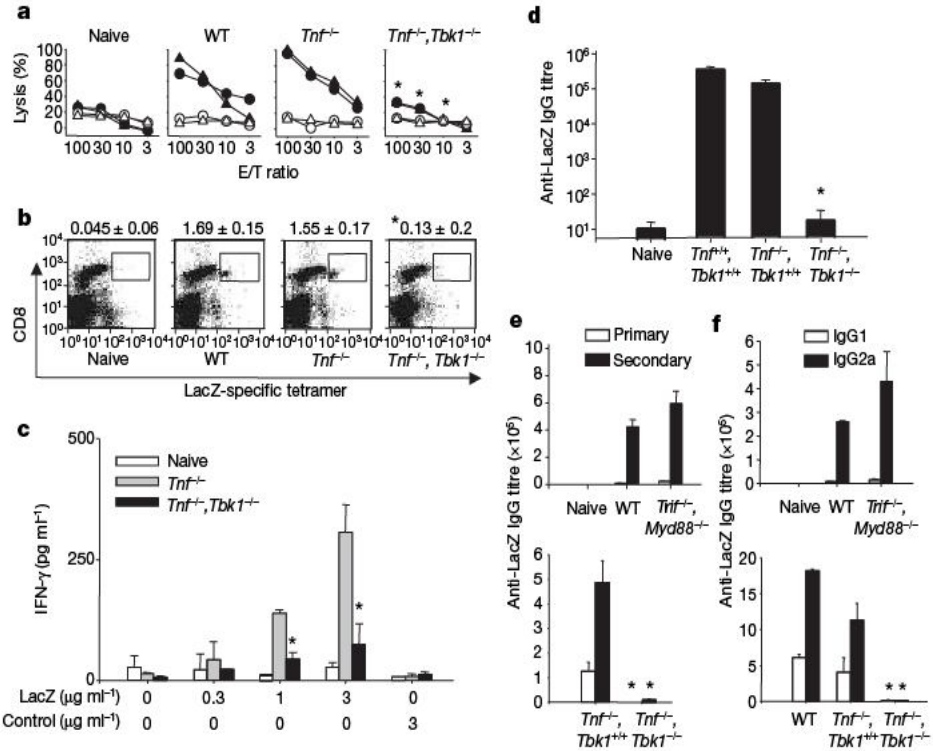


**Figure 2 | *Tbk1*$^{-/-}$ mice failed to elicit antigen-specific T- and B-cell responses after DNA vaccination.** Mice (five per group) deficient for TNF, TNF and TBK1 or MyD88 and TRIF were immunized with a DNA vaccine encoding LacZ protein on days 0 and 28 by intramuscular electroporation, as described in the Methods. Fourteen days after the second immunization, the antigen-specific CTL activity (**a**, representative individual data with (filled symbol) or without (open symbol) LacZ peptide), the frequency of CTL (**b**, positive for LacZ-specific tetramer, in which the number is the average percentage of the gated population ± s.d.) and the LacZ-protein-specific IFN-γ production (**c**) by spleen cells were analysed. LacZ-specific IgG titre (**d**–**f**), including their isotypes, were analysed by ELISA. Data are the averages ± s.d. of five mice per group; *$P < 0.01$ against control (*Tbk1*$^{+/+}$) mice. E/T ratio, effector to target ratio.

(Fig. 2a–c). Moreover, *Tbk1*$^{-/-}$, but not control (*Tnf*$^{-/-}$) or *Myd88*$^{-/-}$,*Trif*$^{-/-}$ (deficient for any known TLR signalling), mice immunized with a different DNA vaccine encoding influenza A virus NP protein also failed to induce IFN-γ-producing spleen cells in response to NP peptides specific to both CD4$^+$ and CD8$^+$ T cells (Supplementary Fig. 4a). To examine whether *Tbk1*$^{-/-}$ T cells are able to induce TLR9-adjuvanted immune responses or not, we immunized control (*Tnf*$^{-/-}$) and *Tbk1*$^{-/-}$ mice with a vaccine consisting of a NP peptide and CpG ODN, the adjuvant effect of which is totally dependent on TLR9 (ref. 25). Both control (*Tnf*$^{-/-}$) and *Tbk1*$^{-/-}$ immunized mice showed antigen-specific IFN-γ production and cytotoxicity of spleen cells (Supplementary Fig. 4b, c), suggesting that the TLR9-mediated adjuvant effects of CpG ODN are intact in the absence of TBK1 and TNF. The normal functions of *Tbk1*$^{-/-}$ T cells and dendritic cells were further confirmed by several assays, because *Tbk1*$^{-/-}$ T cells respond normally to anti-CD3 and anti-CD28 (Supplementary Fig. 5a and b), and *Tbk1*$^{-/-}$ dendritic cells had intact antigen-presenting functions (Supplementary Fig. 5c). These data clearly demonstrate that TBK1-dependent signalling, but not TLR signalling, is essential for DNA-vaccine-induced T-cell responses to the encoded antigen.

We next examined the role of TBK1 in the humoral responses elicited by DNA vaccination. When wild-type and control (*Tnf*$^{-/-}$) mice were immunized with DNA vaccine, their IgG titres against the encoded LacZ protein were significantly augmented in serum; however, titres in *Tbk1*$^{-/-}$ mice were reduced to the level observed in naive mice, nearly 4 log lower than those in control (*Tnf*$^{-/-}$) or wild-type mice (Fig. 2d). This was the case for either primary or secondary immune responses including isotypes (Fig. 2e, f), whereas those in *Myd88*$^{-/-}$ or *Trif*(also called Ticam 1)$^{-/-}$ mice were comparable to those in wild-type mice (Fig. 2e, f). This was not due to malfunction of *Tbk1*$^{-/-}$ B cells, because the levels of total serum IgG, including IgG1 and IgG2a, were at comparable levels to those of wild-type mice (Supplementary Fig. 5d). Taken together, these results strongly suggest that TBK1 is required for the induction of both humoral and cellular immune responses by DNA vaccination *in vivo*.

To elucidate further the intercellular mechanism(s) by which the TBK1-mediated signalling contributes to DNA-vaccine immunogenicity, we next examined the role of TBK1 signalling in haematopoietic and non-haematopoietic cells by transferring the bone marrows of *Tbk1*$^{+/+}$ or *Tbk1*$^{-/-}$ mice into *Tbk1*$^{-/-}$ or *Tbk1*$^{+/+}$ mice on a *Tnf*$^{-/-}$ background, respectively. When *Tbk1*$^{+/+}$ or *Tbk1*$^{-/-}$ chimaeric mice with *Tbk1*$^{-/-}$ bone marrow were immunized with DNA vaccines, the antigen-specific IgG and IFN-γ production were significantly impaired compared with those in *Tbk1*$^{+/+}$ or *Tbk1*$^{-/-}$ chimaeric mice with *Tbk1*$^{+/+}$ bone marrow (Fig. 3a–d). It has been shown that direct transfection of dendritic cells with DNA vaccines can prime both humoral and cellular immune responses to the encoded antigen[26]. To examine whether TBK1-mediated signalling of dendritic cells directly transfected with DNA vaccine is involved in DNA-vaccine-induced immune responses, splenic dendritic cells from wild-type, control (*Tnf*$^{-/-}$) or *Tbk1*$^{-/-}$ mice were transfected with DNA vaccine by electroporation *in vitro*, and were then transferred to naive, control (*Tnf*$^{-/-}$) mice. Serum IgG and IgG2a titers were significantly increased in mice that received wild-type or control (*Tnf*$^{-/-}$) dendritic cells, but not in those that received *Tbk1*$^{-/-}$ (*Tnf*$^{-/-}$) dendritic cells (Fig. 3e), suggesting that TBK1 signalling in dendritic cells is sufficient to prime antigen-specific antibody responses. Taken together, TBK1 signalling in bone-marrow-derived cells, probably dendritic cells, and to a lesser extent that in non-haematopoietic cells, is critical for the optimal humoral response as well as for helper T (Th)1 cytokine production after DNA vaccination.

We also examined the roles of TBK1 in haematopoietic and non-haematopoietic cells, in the induction of antigen-specific CD4$^+$ and

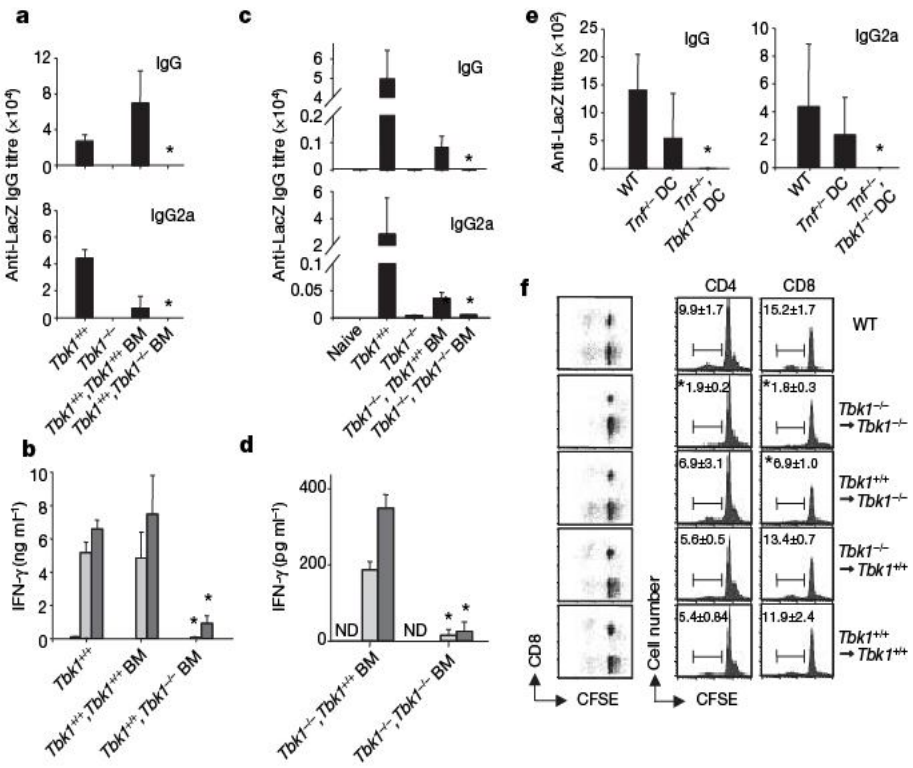


**Figure 3 | Contribution of haematopoietic and non-haematopoietic cells to DNA-vaccine-induced immunogenicity.** Bone marrow (BM) chimaeric mice with TNF-deficient or TNF and TBK1-double-deficient bone marrow were immunized with a DNA vaccine encoding LacZ, as described in the Methods. Fourteen days after the second immunization, sera from the chimaeric mice (TNF-deficient (**a**, **b**) or TNF and TBK1-double-deficient mice (**c**, **d**)) were analysed for LacZ-specific IgG titre (**a**, **c**), and spleen cells were analysed for their antigen-specific IFN-γ production (**b**, **d**) as well as for their antigen-specific CD4$^+$ or CD8$^+$ T-cell proliferation (**f**) in response to LacZ antigen. Splenic dendritic cells from wild-type, control (*Tnf*$^{-/-}$) or *Tbk1*$^{-/-}$ mice were transfected with DNA vaccine by electroporation *in vitro*, and were then transferred to naive, control (*Tnf*$^{-/-}$) mice (**e**). Serum LacZ-specific IgG titre was analysed 3 weeks after dendritic-cell transfer (**e**). Data are the averages ± s.d. of three mice per group (except **f**, which is representative of two experiments); *$P < 0.01$ against wild-type mice. DC, dendritic cell. N.D., not detected.

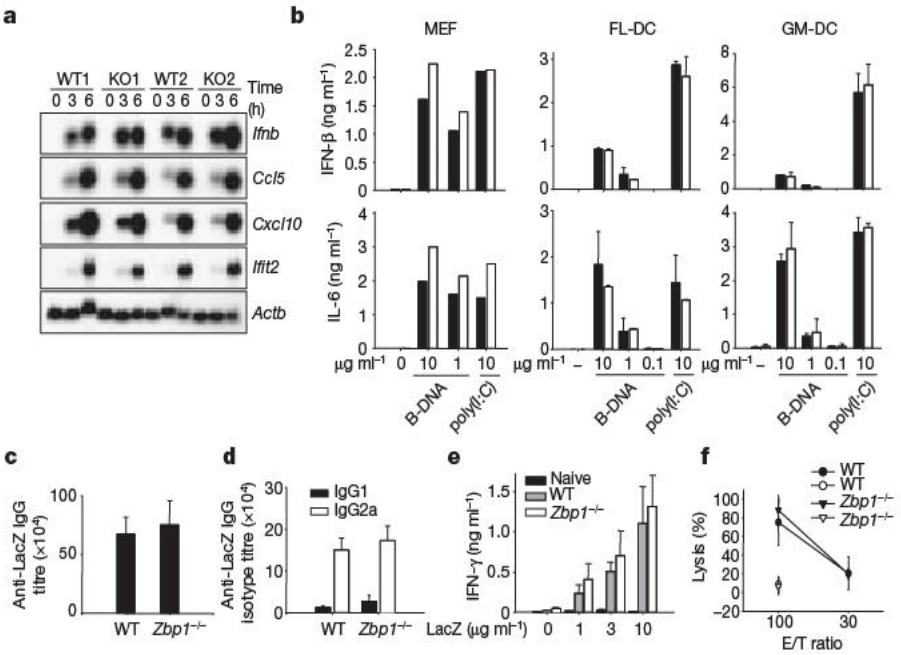


**Figure 4 | Effects of ZBP1 deficiency on the innate immune activation by B-DNA and the adaptive immune responses to DNA vaccine.** MEFs or bone marrow dendritic cells generated by GM-CSF (GM-DC) or Flt3 (Fms-like tyrosine kinase 3) ligand (FL-DC) were stimulated with poly(dA-dT)•poly (dT-dA) by transfection. Three and six hours later, the MEF messenger RNA expression of *Ifnb*, *Ccl5*, *Cxcl10*, *Ifit2* and *Actb* was determined by northern blot analysis (**a**), and IFN-β and IL-6 concentrations at 24 h stimulation were measured by ELISA (**b**, black bars, wild type; white bars, $Zbp1^{-/-}$). Mice lacking ZBP1 were immunized with a DNA vaccine, and two weeks after the second immunization, antigen-specific serum IgG (**c**), IgG1 and IgG2a (**d**) and IFN-γ concentration (**e**) as well as percentage lysis of the immunized mice spleen with (filled symbol) or without (open symbol) LacZ antigen (**f**) were measured. Data are the averages ± s.d. of three mice per group (except **f**, which is representative of two experiments); $^{*}P < 0.01$ against wild-type mice.

$CD8^+$ T cells after DNA vaccination. Wild-type, control ($Tnf^{-/-}$) and $Tbk1^{-/-}$ mice received $Tbk1^{+/+}$ or $Tbk1^{-/-}$ bone marrow cells and were immunized with a DNA vaccine. Proliferation of antigen-specific $CD4^+$ and CD8 $T^+$ cells was analysed by a CFSE (5-(and 6-)carboxyfluorescein diacetate succinimidyl ester)-based division assay using flow cytometry. In wild-type mice immunized with DNA vaccine, a significant number of antigen-specific $CD4^+$ and $CD8^+$ T cells proliferated after five days in response to LacZ antigen (Fig. 3f). Although $Tbk1^{+/+}$ chimaeric mice with $Tbk1^{-/-}$ bone marrow displayed a comparable number of proliferating antigen-specific $CD4^+$ and $CD8^+$ T cells to $Tbk1^{+/+}$ chimaeric mice with $Tbk1^{+/+}$ bone marrow, $Tbk1^{-/-}$ chimaeric mice with $Tbk1^{+/+}$ or $Tbk1^{-/-}$ bone marrow had significantly fewer proliferating antigen-specific $CD8^+$ T cells (Fig. 3f). Interestingly, $Tbk1^{-/-}$ chimaeric mice with $Tbk1^{-/-}$ bone marrow, but not those with $Tbk1^{+/+}$ bone marrow, had significantly fewer proliferating antigen-specific $CD4^+$ T cells (Fig. 3f), suggesting that non-haematopoietic cells (or radiation-resistant cells), but not bone-marrow-derived, most probably haematopoietic cells, are required for optimal antigen-specific $CD8^+$ T-cell proliferation, whereas both are required for proliferation of $CD4^+$ T cells after DNA vaccination.

Our results revealed that the TLR9 ligand activity of plasmid DNA seems minimal for its adjuvant effects, and that the double-stranded B-form of plasmid DNA might be the critical adjuvant element for DNA vaccine, especially when introduced into the cytoplasm and/or nucleus by transfection such as electroporation. We carefully excluded possibilities that RNA generated during DNA vaccination acts as an adjuvant by activating TBK1-dependent signalling, because mice deficient for TRIF, MyD88 and IPS-1 are essential for TLR3-, TLR7/8- and RIG-I/MDA5-mediated RNA recognition, respectively, were intact in inducing DNA vaccine immunogenicity (Supplementary Figs 2 and 6). We also evaluated a possibility of ZBP1 (renamed as DAI in ref. 4 which was recently demonstrated *in vitro* as a candidate for a B-DNA receptor[4], by generating its knockout mice (Supplementary Fig. 7). The results *in vitro* and *in vivo*, however, showed that ZBP1 was not essential for either innate or adaptive responses to B-DNA or DNA vaccination, respectively (Fig. 4). Mouse embryonic fibroblasts (MEFs), two type of bone-marrow dendritic cells and macrophages responded to B-DNA and plasmid DNA as well as DNA virus infection normally to produce type-I IFNs, IL-6 and the other IFN-inducible chemokines, evaluated by northern blot, PCR with reverse transcription (RT–PCR) and ELISA (Fig. 4a, b and data not shown). In addition, ZBP1 was dispensable for inducing DNA vaccine immunogenicity including both T and B cells specific to the encoded antigen (Fig. 4c–f). Thus, ZBP1 is dispensable for both innate and adaptive immune responses to B-DNA and DNA vaccine, respectively, although its redundant role(s) is not formally excluded.

The importance of TBK1-mediated innate immune signalling for adjuvant effect, possibly through type-I IFNs, has been implicated because TRIF-dependent signalling was the major contributor to the adjuvant activity of monophosphoryl lipid A[5], and co-administration of the *Irf3*, *Irf7* or *Trif* gene as a genetic adjuvant for a DNA vaccine augmented the immunogenicity[17,27]. It will be of interest to investigate whether activation of TBK1-dependent pathway is involved in the immunogenicity of the other vaccines and to develop novel vaccine adjuvants that activate the TBK1-dependent signalling pathway. Although further studies are needed to clarify the factors including a potential DNA sensor(s) that mediates DNA-activated, TBK1-mediated innate immune activations towards adaptive immune responses or, ultimately, memory, our results may provide insights into the molecular and cellular mechanisms by which DNA vaccines trigger innate and adaptive immune responses to the encoded antigen.

## METHODS SUMMARY

**Mice, cells and reagents.** Mutant mice lacking TNF, TBK1, IKK-i (encoded by $Ikbke^{-/-}$), TLR9, MyD88, TRIF, IFN-αβR (encoded by $Ifnr2^{-/-}$) or IPS-1, either on a 129/Ola × C57/BL6 or on a C57/BL6 background, have been described previously[20,28]. Mice lacking ZBP1 (also known as DLM-1 or DAI) were generated as described in Supplementary Fig. 7a and in the Methods. Spleen cells, MEFs and dendritic cells (GM-DCs or FL-DCs) were prepared as described previously[20]. Cells were stimulated in the presence of the indicated stimuli, and supernatants or total RNAs were collected for cytokine ELISA or for northern blot or RT–PCR, respectively, performed as described previously[20,24].

**DNA vaccination.** Immunization of mice (3–5 mice per group) with a DNA vaccine encoding LacZ or influenza A virus NP proteins was performed by intramuscular (i.m.) electroporation (100 μg per mouse), as described previously[17]. Mice were immunized twice, on days 0 and 28, followed by immunological assays two weeks after the second immunization unless otherwise indicated. In some experiments, splenic dendritic cells were electroporated with DNA vaccine *in vitro* and transferred intravenously into naive mice as described previously[29]. In some experiments, bone marrow was transferred approximately 1–2 months before DNA immunization as described previously[30]. All animal experiments were approved by the institutional animal care and welfare committee, and the mice were treated in accordance with the animal care guidelines of the Research Institute for Microbial Diseases, Osaka University, Japan.

**Measurements of LacZ- or NP-specific immune responses.** The serum anti-LacZ antibody titre was measured by ELISA as described previously[17]. A cytotoxic T lymphocyte (CTL) assay was performed as described previously[17]. Antigen (LacZ or NP)-specific IFN-γ production was analysed as described previously[17,28]. The number of Lac-Z-specific $CD8^+$ T cells was also measured using phycoerythrin (PE)-conjugated H-$2D^b$/LacZ(96–103) tetramer reagent (MBL)[28]. To analyse the proliferation of $CD4^+$ and $CD8^+$ T cells, spleen cells were stained with CFSE (Molecular Probes) and were cultured in the presence of LacZ protein (10 μg $ml^{-1}$) for 5 days. Spleen cells were stained with anti-CD4, anti-T cell receptor β (anti-TCRβ) and anti-CD8 antibody and analysed with a FACS Calibur instrument (BD) using CellQuest software (BD).

**Statistical analysis.** Differences between groups were analysed for statistical significance by the Student's *t*-test or ANOVA, using SigmaStat 3.0 software.

**Full Methods** and any associated references are available in the online version of the paper at www.nature.com/nature.

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**Supplementary Information** is linked to the online version of the paper at www.nature.com/nature.

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**Author Contributions** K.J.I., C.C. and S.A. designed the research and analysed data. K.J.I., S.K. and C.C. performed most experiments. T.K. generated ZBP-1-deficient mice and performed the related experiments. K.M. and O.T. performed the bone-marrow-transfer experiments. S.U., T.K. and H.K. provided mutant mice. F.T. provided critical materials and advice. K.J.I., C.C. and S.A. prepared the manuscript.

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LETTERS

# *Drosophila* Pgc protein inhibits P-TEFb recruitment to chromatin in primordial germ cells

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Germ cells are the only cells that transmit genetic information to the next generation, and they therefore must be prevented from differentiating inappropriately into somatic cells[1]. A common mechanism by which germline progenitors are protected from differentiation-inducing signals is a transient and global repression of RNA polymerase II (RNAPII)-dependent transcription[1]. In both *Drosophila* and *Caenorhabditis elegans* embryos, the repression of messenger RNA transcription during germ cell specification correlates with an absence of phosphorylation of Ser 2 residues in the carboxy-terminal domain of RNAPII (hereafter called CTD)[2], a critical modification for transcriptional elongation[3]. Here we show that, in *Drosophila* embryos, a small protein encoded by *polar granule component* (*pgc*) is essential for repressing CTD Ser 2 phosphorylation in newly formed pole cells, the germline progenitors. Ectopic Pgc expression in somatic cells is sufficient to repress CTD Ser 2 phosphorylation. Furthermore, Pgc interacts, physically and genetically, with positive transcription elongation factor b (P-TEFb), the CTD Ser 2 kinase complex, and prevents its recruitment to transcription sites. These results indicate that Pgc is a cell-type-specific P-TEFb inhibitor that has a fundamental role in *Drosophila* germ cell specification. In *C. elegans* embryos, PIE-1 protein segregates to germline blastomeres, and is thought to repress mRNA transcription through interaction with P-TEFb[4–7]. Thus, inhibition of P-TEFb is probably a common mechanism during germ cell specification in the disparate organisms *C. elegans* and *Drosophila*.

*pgc* RNA, a component of the *Drosophila* germ plasm[8], has been implicated in the repression of CTD Ser 2 phosphorylation in early pole cells[9,10]. However, the mechanism underlying *pgc*-mediated transcriptional repression has been unknown. Initial characterization of its nucleotide sequence suggested that *pgc* RNA acts as a non-coding RNA[8]. Nevertheless, we have noticed that an AUG triplet, beginning at nucleotide 117 of the 0.7-kilobase (kb) transcript, is in a favourable context to serve as a translation initiation site[8]. Completion of the *Drosophila* genomic sequence revealed an error in our original manual sequencing in a region where a strong stem structure can form (an additional C exists between nucleotides 178 and 179). In the revised *pgc* sequence, the open reading frame (ORF) beginning at nucleotide 117 is capable of encoding a 71-amino-acid polypeptide (Fig. 1a). We found that this polypeptide sequence is

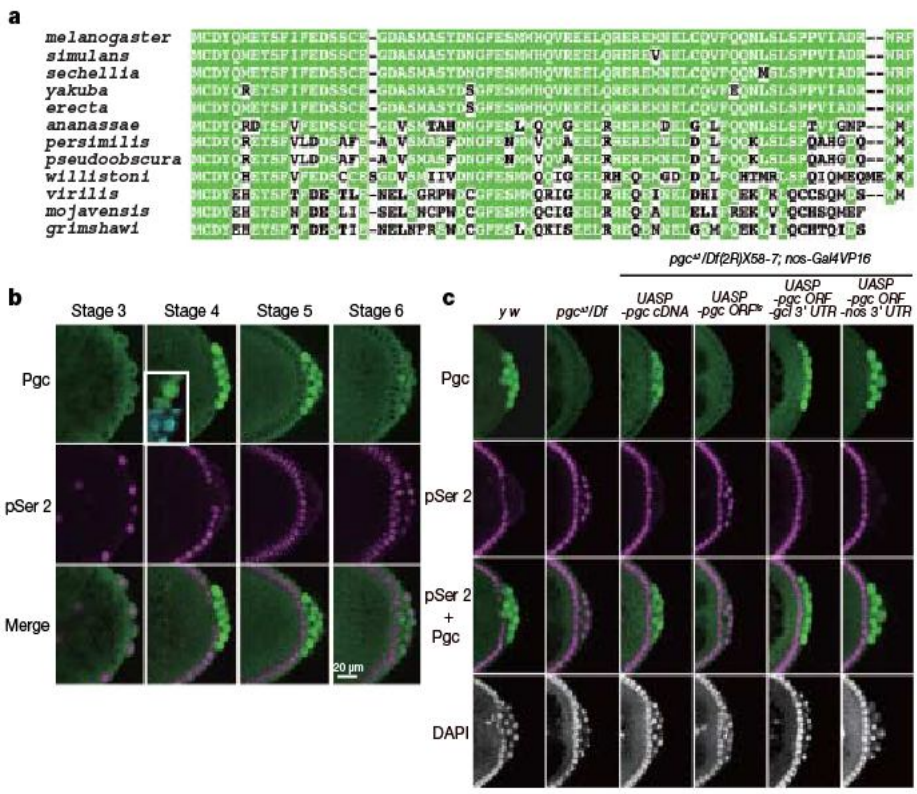


**Figure 1 | Pgc expression in pole cells is essential for the repression of CTD Ser 2 phosphorylation.** **a**, Pgc sequences of 12 *Drosophila* species. Conserved amino acid residues are highlighted by green shading. Note that no apparent Pgc orthologues can be found in other animal groups. **b**, Pgc expression in pole cells is complementary to pSer 2. Panels show the posterior pole of wild-type embryos immunostained for Pgc (green) and pSer 2 (magenta). Nuclei were counter-stained with 4,6-diamidino-2-phenylindole (DAPI; cyan). Pgc is concentrated in the nucleus (inset). **c**, Posterior poles of stage-4 embryos immunostained for pSer 2 (magenta) and Pgc (green). Nuclei were counter-stained with DAPI. Maternal genotypes are indicated. Note that *pgc ORF^fs* RNA accumulates normally in the germ plasm (Supplementary Fig. 2).

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conserved in 12 *Drosophila* species for which genomes have been sequenced (Fig. 1a), although no homologous sequences have been identified in other animal groups, even in dipteran insects.

Antibodies raised against the 71-amino-acid polypeptide showed immunoreactivity in wild-type pole cells, but not in pole cells lacking *pgc* (Fig. 1b, c). The signals were first detected in pole cells at stage 4, when pole cells are formed. Although *pgc* RNA is detectable in pole cells until mid-embryogenesis[8], the Pgc immunoreactivity in pole cells dropped markedly at stage 6. We did not detect the Pgc signal in somatic cells. These dynamic patterns of Pgc expression suggest that *pgc* RNA translation and Pgc protein stability are regulated. Notably, double staining of wild-type embryos for Pgc and CTD phosphorylated at Ser 2 (pSer 2) revealed that the Pgc expression in pole cells was complementary to pSer 2 (Fig. 1b).

To investigate the function of *pgc* in repressing CTD Ser 2 phosphorylation, we generated $pgc^{\Delta 1}$, a chromosomal null for *pgc* (Supplementary Fig. 1). Homozygous or hemizygous $pgc^{\Delta 1}$ females were fertile, and embryos from $pgc^{\Delta 1}$ mothers (hereafter termed $pgc^{-}$ embryos) formed normal numbers of pole cells (Fig. 1c, Supplementary Fig. 2 and data not shown). However, $pgc^{-}$ pole cells failed to repress CTD Ser 2 phosphorylation during stages 4–5 (Fig. 1c). These pole cells degenerated from stage 10 onwards, and few or no pole cells coalesced into the gonads (Supplementary Fig. 2). Consequently, about 80% of the $pgc^{-}$ embryos developed into sterile adults. These defects were rescued by the expression of intact *pgc* RNA, but not a frame-shift version of *pgc* RNA ($pgc\ ORF^{fs}$), during oogenesis (Fig. 1c and Supplementary Figs 2 and 3). We next made hybrid transgenes in which the *pgc* ORF was fused with the *nanos* (*nos*) or *germ cell-less* (*gcl*) 3′ UTR, which contains RNA localization signals that mediate accumulation in the germ plasm. Expression of these fusion genes during oogenesis in $pgc^{\Delta 1}$ hemizygotes promoted Pgc expression in early pole cells and repressed CTD Ser 2 phosphorylation (Fig. 1c). Pole cell death was also rescued in these embryos (Supplementary Fig. 2 and data not shown), which developed into fertile adults. These results indicate that Pgc protein is essential for the repression of CTD Ser 2 phosphorylation in pole cells

When Pgc was misexpressed at the anterior of the blastoderm embryos, CTD Ser 2 phosphorylation and zygotic expression of a *hunchback* (*hb*)-*lacZ* reporter was repressed in somatic cells where ectopic Pgc was detected (Fig. 2 and Supplementary Fig. 4). Furthermore, Pgc expression in *Drosophila* S2 cells repressed CTD Ser 2 phosphorylation (Supplementary Fig. 5). These results demonstrate that Pgc can prevent CTD Ser 2 phosphorylation even in somatic cells, and further suggest that the target of Pgc action is a general component of the RNAPII-dependent transcription machinery.

Positive transcription elongation factor-b (P-TEFb) is responsible for CTD Ser 2 phosphorylation *in vivo*[3,11–13], making it a candidate Pgc target. *Drosophila* P-TEFb consists of a kinase subunit, Cdk9, and

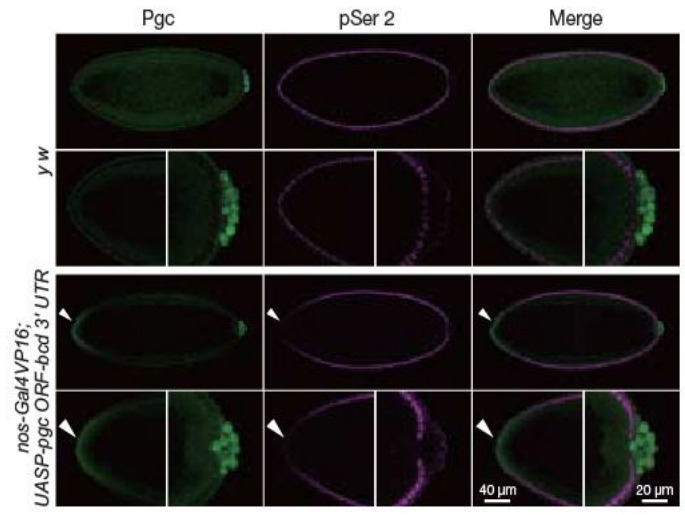


**Figure 2 | Pgc is sufficient to repress CTD Ser 2 phosphorylation in somatic cells.** Embryos were immunostained for Pgc (green) and pSer 2 (magenta). High magnifications of anterior and posterior parts of the embryos are also shown. To misexpress Pgc at the anterior somatic cell region, a hybrid gene, in which the *pgc* ORF was fused with the *bicoid* (*bcd*) anterior localization signal, was expressed in oogenesis. In embryos expressing *pgc-bcd 3′ UTR* mRNA, CTD Ser 2 phosphorylation was repressed in regions expressing ectopic Pgc (arrowheads). Many embryos (50–80%) expressing the *pgc ORF-bcd 3′ UTR* transgene died with variable defects, but we never observed a bicaudal phenotype, suggesting that anterior misexpression of Pgc is incapable of recruiting germ plasm components required for posterior development.

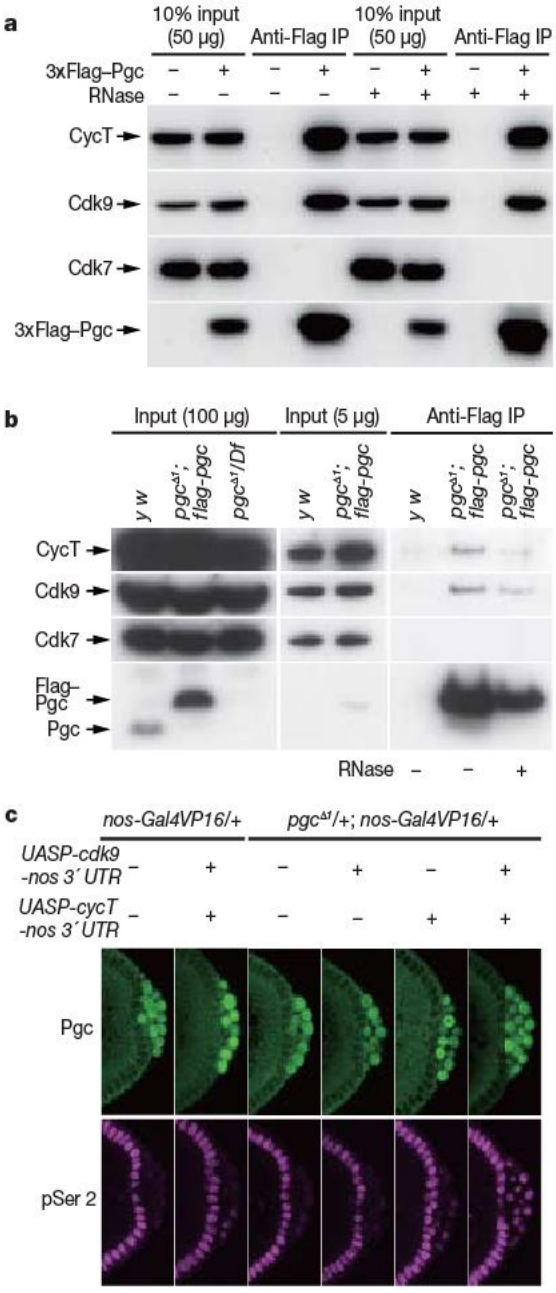


**Figure 3 | Pgc interacts, physically and genetically, with P-TEFb. a**, Cdk9 and CycT, but not Cdk7, were co-immunoprecipitated with Pgc from the lysates of S2 cells expressing 3×Flag–Pgc. **b**, Lysates of *y w* or $pgc^{-}$ embryos expressing the *flag–pgc* transgene were immunoprecipitated with anti-Flag antibody, and bound proteins were analysed by western blotting. **c**, P-TEFb was overexpressed in pole cells by expressing *cycT-nos 3′ UTR* and *cdk9-nos 3′ UTR* transgenes in oogenesis. Overexpression of P-TEFb in pole cells caused precocious CTD pSer 2 phosphorylation (magenta), even in the presence of Pgc expression (green). This precocious CTD pSer 2 phosphorylation was strongly induced when P-TEFb was overexpressed in the $pgc^{\Delta 1}$ heterozygous background.

a regulatory subunit, Cyclin T (CycT)[13–15]. Supporting a link between P-TEFb and Pgc, both Cdk9 and CycT were co-immunoprecipitated with 3×Flag–Pgc from S2 cell lysates, but another CTD kinase, Cdk7 (a component of TFIIH that preferentially phosphorylates CTD Ser 5; ref. 3), was not (Fig. 3a). A reciprocal immunoprecipitation also confirmed a specific interaction between Pgc and P-TEFb (Supplementary Fig. 6). To examine their interaction in pole cells, we used a Flag-tagged *pgc* transgene that fully rescued the $pgc^{\Delta 1}$ mutant. Lysates from pools of embryos enriched for stages 4–5 were immunoprecipitated with anti-Flag antibody. Cdk9 and CycT were specifically co-immunoprecipitated with Flag–Pgc from the lysates of the transgenic embryos but not from control lysates (Fig. 3b). RNase treatment of lysates did not affect the Pgc–P-TEFb interaction (Fig. 3a, b). These results demonstrate that Pgc forms a complex with both CycT and Cdk9.

Pull-down assay showed that maltose-binding protein (MBP)–Pgc fusion protein, but not control MBP, interacted with the P-TEFb complex *in vitro* (Supplementary Fig. 7a). To examine which subunit of P-TEFb interacts with Pgc, *in-vitro*-synthesized Cdk9 or CycT was individually tested for pull-down assay. Cdk9, but not CycT, was specifically pulled down by MBP–Pgc (Supplementary Fig. 7b, c), indicating that Cdk9 alone is sufficient for the direct interaction with Pgc *in vitro*. Taken together, these results suggest that Pgc can form a ternary complex with Cdk9 and CycT.

To investigate the significance of the interaction between Pgc and P-TEFb *in vivo*, we overexpressed P-TEFb in pole cells. Although overexpression of P-TEFb failed to induce CTD Ser 2 phosphorylation in pole cells at stage 4, when Pgc expression is highest (Fig. 1b), it caused precocious CTD Ser 2 phosphorylation in a few pole cells at stage 5 (Fig. 3c). Furthermore, precocious CTD Ser 2 phosphorylation was strongly induced when both transgenes were expressed in the $pgc^{\Delta 1}$ heterozygous background (Fig. 3c). Many pole cells in these embryos degenerated during embryogenesis, and the gonads often included few or no pole cells (Supplementary Table 1). The overexpression of P-TEFb in pole cells promoted the ectopic expression of somatic genes that are misexpressed in $pgc^{-}$ pole cells (Supplementary Fig. 8)[9,10]. By contrast, no ectopic expression of *even skipped* (*eve*) or *fushi tarazu* (*ftz*), which are not misexpressed in $pgc^{-}$ pole cells[9], was detected in P-TEFb-overexpressing pole cells (data not shown). Thus, overexpression of P-TEFb in pole cells mimics *pgc* mutant phenotypes in a *pgc* dosage-dependent manner. These data demonstrate that Pgc represses CTD Ser 2 phosphorylation in pole cells by interfering with P-TEFb action.

Pgc alone was unlikely to inhibit the kinase activity of P-TEFb, as MBP–Pgc, which interacted with P-TEFb *in vitro* (Supplementary Fig. 7), did not affect CTD phosphorylation by P-TEFb in an *in vitro* kinase assay (Supplementary Fig. 9). The recruitment of P-TEFb to paused promoter regions is crucial for the productive elongation of most nascent transcripts[13]. We therefore examined the effects of Pgc expression in salivary glands on the distribution of P-TEFb. In control polytene chromosomes, Cdk9 and CycT co-localized at numerous sites, which were also positive for pSer 5, a marker for active transcription[3] (Fig. 4a), indicating that P-TEFb is recruited to active promoter regions as reported[16]. By contrast, when Pgc was expressed in salivary glands, the P-TEFb signals on the polytene chromosomes were severely decreased; instead, P-TEFb was mostly detected on the cell debris (arrowheads in Fig. 4a). Western blot analysis showed that Pgc expression caused an ~40% reduction in the level of pSer 2, whereas the levels of Cdk9 and CycT were virtually unchanged (Fig. 4b). Pgc expression in salivary glands also caused a reduction in the levels of pSer 5 (to ~65%), similar to the effects of RNA-interference-mediated *cdk9* knockdown in *Drosophila* larvae[17]. These results suggest that Pgc sequesters P-TEFb and prevents its recruitment to active promoters.

We next examined the effects of Pgc expression on the recruitment of P-TEFb to transcription sites by chromatin immunoprecipitation (ChIP) assay. Consistent with previous reports[12,18], heat-shock treatment promoted the rapid recruitment of CycT and Cdk9 to the *Hsp70* and *Hsp27* gene regions in control S2 cells (Fig. 4c). By contrast, Pgc expression caused a significant reduction in the levels of both CycT and Cdk9 on these genes after heat shock (Fig. 4c; $P < 0.01$). RNAPII distribution on these gene regions was not significantly affected by Pgc expression (Fig. 4c; $P > 0.4$), similar to the effects of P-TEFb inactivation in *Drosophila* cells[12]. These results support the idea that Pgc represses CTD Ser 2 phosphorylation by inhibiting the recruitment of P-TEFb to transcription sites.

P-TEFb is a key regulator of RNAPII-dependent transcription of most cellular genes[3,13–15] as well as HIV-1 transcription and replication[13,19,20]. In mammals, P-TEFb is inhibited by the 7SK RNA–HEXIM1 complex, which suppresses the Cdk9 kinase activity and prevents P-TEFb from binding to transcription templates[12,21–24]. Pgc acts through an analogous—but not identical—mechanism, as Pgc

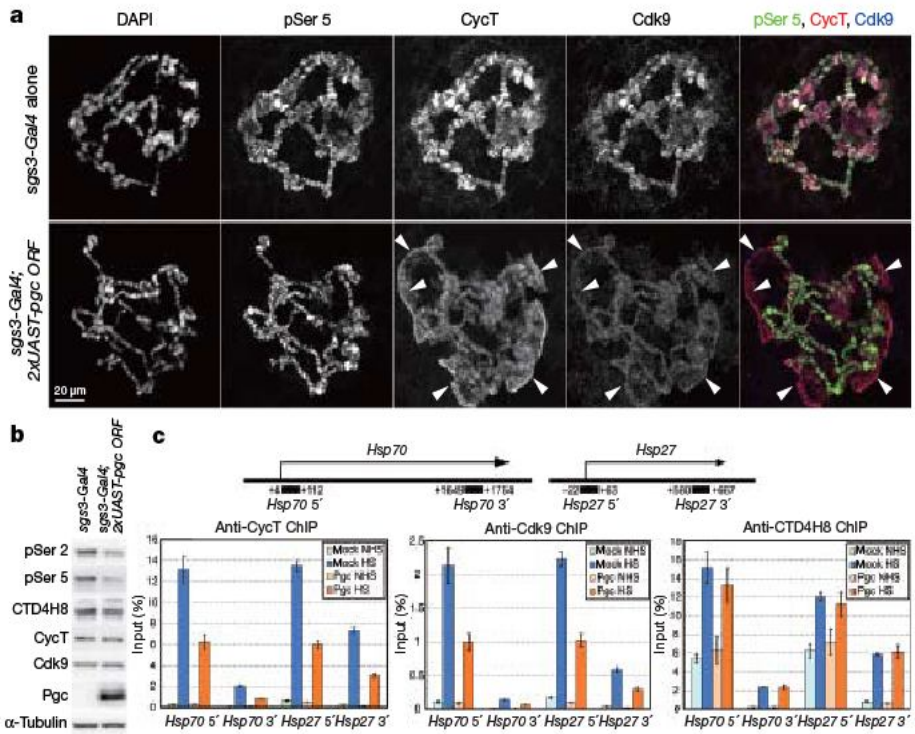


**Figure 4 | Pgc prevents P-TEFb recruitment.** **a**, Salivary gland polytene chromosome squashes prepared from third-instar larvae carrying either the *sgs3-Gal4* driver alone or *sgs3-Gal4* plus two copies of *UAST-pgc* ORF transgenes immunostained for pSer 5, CycT and Cdk9, and counter-stained with DAPI. Arrowheads indicate P-TEFb signals on cell debris in the Pgc-expressing salivary gland squash. **b**, Western blot analysis of salivary gland lysates from *sgs3-Gal4* or *sgs3-Gal4*; *2×UAST-Pgc* third-instar larvae. **c**, Real-time PCR analyses of ChIP experiments on the *Hsp70* and *Hsp27* gene regions in heat-shocked (HS) or non-heat-shocked (NHS) S2 cell transfectants. The proportion of Pgc-expressing S2 cells after $CuSO_4$ induction was about 70%. Each result shows an average of at least three independent experiments with the standard error of the mean.

alone failed to inhibit the kinase activity of P-TEFb *in vitro* (Supplementary Fig. 9). Furthermore, 7SK RNA is unique to vertebrates[13], and the Pgc–P-TEFb interaction is RNase insensitive (Fig. 3a, b). We speculate that additional Pgc-like P-TEFb inhibitors may exist in somatic cells. In *C. elegans* germline blastomeres, PIE-1 is proposed to prevent CTD Ser 2 phosphorylation through interaction with CycT[6,7]. Thus, P-TEFb seems to be a common regulatory target for the repression of mRNA transcription during germ cell specification in both flies and nematodes. Despite their analogous functions, Pgc and PIE-1 are unrelated in sequence and therefore must have arisen independently in evolution. This is consistent with the hypothesis that germ cell specification by the maternally inherited germ plasm evolved independently among diverse animal groups[25]. The inhibition of somatic transcriptional programmes is also crucial for the establishment of mouse germ cells, which are specified through an epigenetic mechanism[1,26–28]. Whether P-TEFb is targeted in mammalian germ cell progenitors as well will be an interesting question in the future.

## METHODS SUMMARY

**Fly strains and transgenic constructs.** The fly strains used in this study are described in FlyBase, unless otherwise noted. *rF139* is a homozygous-viable *P[ry$^+$ PZ]* insertion that is located ~13 kb distal to the 5′ side of the *pgc* locus and was obtained from the Berkeley *Drosophila* Genome Project (BDGP). Imprecise excision of the *rF139* transposon generated a line (*pgc$^{\#1}$*) in which an ~15-kb genomic region containing the entire *pgc* locus was deleted. The *pgc$^{\#1}$* chromosome also has the deletion of an essential gene, *gp150*, which was subsequently rescued by introducing an ~17.5-kb genomic fragment containing the *gp150* locus, thus generating *pgc$^{\Delta 1}$*, a chromosomal null for *pgc* and *T3dh* (Supplementary Fig. 1). *pgc* cDNA was amplified by PCR and cloned into pUASP to generate *UASP-pgc* cDNA. *UASP-pgc ORF$^{fs}$* contains a 2-base deletion at the fifth codon. The full-length *nos* and *gcl* 3′ UTRs and the *Eco*RV/*Stu*I fragment of the *bcd* 3′ UTR region[29] were used to create chimaeric gene constructs.

**Immunostaining and *in situ* hybridization.** Immunostaining and *in situ* hybridization of embryos were performed using standard procedures. To detect Pgc and pSer 2 signals, the vitelline membranes of fixed embryos were hand peeled, as the reactivity of these antibodies was found to be sensitive to methanol, which is generally used for devitellinization. Salivary glands from climbing third-instar larvae cultured at 27 °C were fixed in 2% paraformaldehyde (PFA) in PBS containing 0.1% Triton-X100 for 30 s followed by 45% acetic acid/1.85% PFA for 5 min before being squashed onto coated microscope slides. Polytene chromosomes were pre-treated with PBS containing 0.1% SDS for 10 min before antibody staining. The specificities of newly raised anti-Cdk9 and anti-CycT antibodies were examined by western blot analyses of lysates from S2 cells depleted of Cdk9 or CycT by RNA interference (Supplementary Fig. 10).

**Full Methods** and any associated references are available in the online version of the paper at www.nature.com/nature.

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**Supplementary Information** is linked to the online version of the paper at www.nature.com/nature.

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**Author Contributions** K.H.-N., P. L. and A.N. conceived and designed the experiments. K.H.-N., H.S.-N., A T. and A. N. performed the experiments and generated all the figures. P.L. and A.N. wrote the paper.

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LETTERS

# Cell cycle control of centromeric repeat transcription and heterochromatin assembly

Ee Sin Chen[1], Ke Zhang[1], Estelle Nicolas[1], Hugh P. Cam[1], Martin Zofall[1] & Shiv I. S. Grewal[1]

**Heterochromatin in eukaryotic genomes regulates diverse chromosomal processes including transcriptional silencing[1]. However, in *Schizosaccharomyces pombe* RNA polymerase II (RNAPII) transcription of centromeric repeats is essential for RNA-interference-mediated heterochromatin assembly[2–5]. Here we study heterochromatin dynamics during the cell cycle and its effect on RNAPII transcription. We describe a brief period during the S phase of the cell cycle in which RNAPII preferentially transcribes centromeric repeats. This period is enforced by heterochromatin, which restricts RNAPII accessibility at centromeric repeats for most of the cell cycle. RNAPII transcription during S phase is linked to loading of RNA interference and heterochromatin factors such as the Ago1 subunit of the RITS complex[6] and the Clr4 methyltransferase complex subunit Rik1 (ref. 7). Moreover, Set2, an RNAPII-associated methyltransferase[8] that methylates histone H3 lysine 36 at repeat loci during S phase, acts in a pathway parallel to Clr4 to promote heterochromatin assembly. We also show that phosphorylation of histone H3 serine 10 alters heterochromatin during mitosis, correlating with recruitment of condensin that affects silencing of centromeric repeats. Our analyses suggest at least two distinct modes of heterochromatin targeting to centromeric repeats, whereby RNAPII transcription of repeats and chromodomain proteins bound to methylated histone H3 lysine 9 mediate recruitment of silencing factors. Together, these processes probably facilitate heterochromatin maintenance through successive cell divisions.**

The formation of heterochromatin, which involves methylation of histone H3 at lysine 9 (H3K9me) by Clr4/Suv39h and subsequent recruitment of chromodomain proteins such as Swi6/HP1, is essential for a variety of cellular functions[1]. In *S. pombe*, a broad distribution of heterochromatin is observed at pericentromeric regions, subtelomeres and the silent mating-type locus[3]. These loci share a common feature: each contains *dg* and/or *dh* repeats that serve as RNA interference (RNAi)-dependent heterochromatin nucleation centres[1,9].

Because *S. pombe* cells have an extended G2 phase[10], asynchronous cultures used to perform most heterochromatin analyses contain predominantly G2 cells. Consequently, heterochromatin regulation in the context of the cell cycle remains largely unexplored. We assessed whether heterochromatin is dynamically regulated during the cell cycle, thus providing a 'window of opportunity' during which repeats might be preferentially transcribed. Temperature-sensitive *cdc25-22* mutant cells were arrested at the G2/M boundary and released to grow synchronously[11]. Cell cycle progression was monitored by septation index (the percentage of cells with a septum), which peaks during S phase[11]. Polymerase chain reaction with reverse transcription (RT–PCR) analyses of transcripts derived from *dh* elements present at centromeres, mating-type locus (*cenH*) and subtelomeric loci (*SPAC212.11*) (Fig. 1a) revealed elevated levels of transcripts during S phase (Fig. 1b), coinciding with the peak of the septation index.

Centromeric repeats are transcribed in both directions[2]. Reverse strand transcription can occur even in the presence of heterochromatin but forward strand transcription is repressed by chromatin-based mechanisms[2,12]. To determine whether the two strands are differentially regulated during the cell cycle, we performed strand-specific RT–PCR. The forward transcripts showed preferential accumulation during S phase (Supplementary Fig. 1). Specific induction

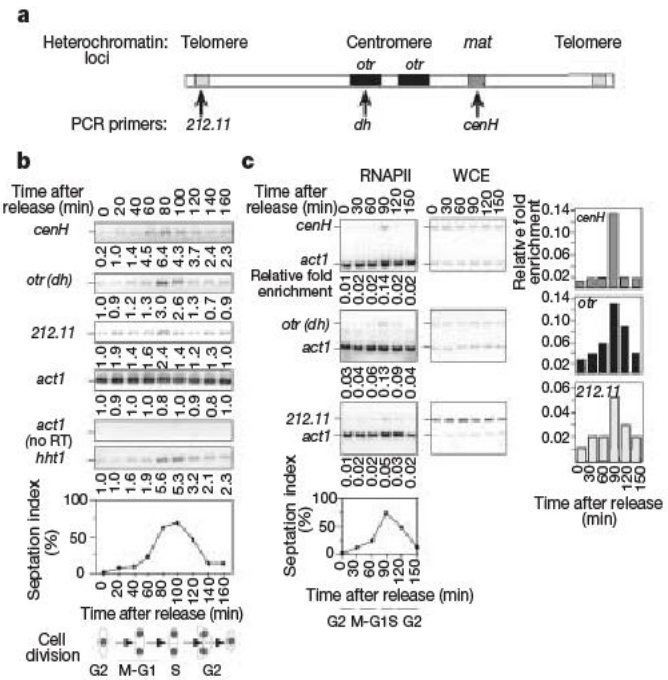


**Figure 1 | Levels of heterochromatin-derived transcripts and RNAPII occupancy peak during S phase.** **a**, Schematic representation of the three heterochromatic loci analysed. Shown are: *cenH*, a *dg/dh*-like element located at a silent *mat* region (grey); *212.11*, the *SPAC212.11* gene possessing homology to *dh*, located near telomeres (light grey); and *dh* repeats at the outer (*otr*) centromere (black). **b**, Heterochromatic repeat transcript levels are enriched during S phase. RT–PCR of RNA isolated from synchronized *cdc25-22* cells. Transcript derived from an S-phase-specific *hht1* gene encoding histone H3, and constitutively expressed *act1*, served as controls. No RT, no reverse transcription. Numbers indicate relative fold increase in transcript levels. **c**, RNAPII preferentially binds heterochromatic repeats during S phase. Localization of the RNAPII subunit Rpb1 was assessed by ChIP. ChIP and whole-cell extract (WCE) DNA samples were subjected to multiplex PCR. Relative fold enrichments depicting the ratios of the signals at repeat loci relative to *act1*, between ChIP and WCE, are shown beneath each lane, and in bar graphs.

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of forward transcripts probably has implications for assembly of heterochromatic structures (see below).

Accumulation of transcripts during S phase suggests that heterochromatin might be more accessible to the transcriptional machinery during this phase. Consistent with this idea, RNAPII occupancy at the repeat loci peaked during S phase (Fig. 1c and Supplementary Fig. 2a). Preferential RNAPII binding correlated with elevated levels of histone H3 acetylation (Supplementary Fig. 3), which also coincided with peaks of *dh* transcript levels (Fig. 1b).

We next used *Δclr4*, a heterochromatin-defective strain lacking H3K9me[13], to explore the role of heterochromatin in regulating the accessibility of RNAPII to repeats during different cell cycle phases. High levels of RNAPII as well as transcripts associated with repeat elements persist throughout the cell cycle in *Δclr4* cells, which contrasts with the S-phase-specific increase in wild-type cells (Fig. 2a, b).

Heterochromatin has been suggested to have little or no effect on RNAPII occupancy at certain repeat sequences, indicating differential effects by heterochromatin on RNAPII accessibilities across centromeric domains[14]. To explore in depth the impact of heterochromatin on RNAPII occupancy across domains containing *dg/dh* repeats, we performed chromatin immunoprecipitation (ChIP)–chip analyses using wild type and *Δclr4* cells. In wild-type cells, levels of RNAPII across heterochromatic domains were markedly lower than the surrounding euchromatic regions (Fig. 2c). Loss of Clr4 resulted in a marked increase in RNAPII occupancy at heterochromatic repeats. These data strongly argue in favour of heterochromatin inhibiting RNAPII occupancy at centromeric repeats.

Heterochromatin limiting RNAPII binding to centromeric repeats suggests that heterochromatic structures might be altered to facilitate RNAPII access to repeats during S phase. Indeed, a marked reduction in H3K9me and Swi6 levels at centromeric repeats was observed as cells entered mitosis (Fig. 3a, b and Supplementary Fig. 2b) and persisted until S phase but increased gradually thereafter. Decreased levels of heterochromatin during mitosis correlated with the preferential enrichment of histone H3 serine 10 phosphorylation (H3S10ph) (Fig. 3a), which has been shown to antagonize the binding of chromodomain proteins to H3K9me[15–17]. Thus, large-scale heterochromatin remodelling is coupled to H3S10ph. However, it should be noted that low levels of chromatin-bound Swi6 persist during mitosis, which has important implications for the functions and propagation of heterochromatic structures[1,18].

The inverse relationship between the localization of Swi6 and H3S10ph was also observed at other heterochromatic loci (Supplementary Fig. 4). However, the decreased levels of heterochromatic components during mitosis did not correlate with increased RNAPII levels at repeat loci (Fig. 1). Instead, H3S10ph-coupled reduction of Swi6 coincided with the recruitment of condensin, implicated in mitotic chromosome condensation[19]. Cut3, a condensin subunit, displays a similar enrichment pattern at heterochromatin to that of H3S10ph, although Cut3 delocalizes from chromatin around G1/S, just before the time when levels of RNAPII peaked (Fig. 3c). The timing of condensin binding corresponds with the mitosis-specific nuclear localization of condensin[20], and concurs with suggestions

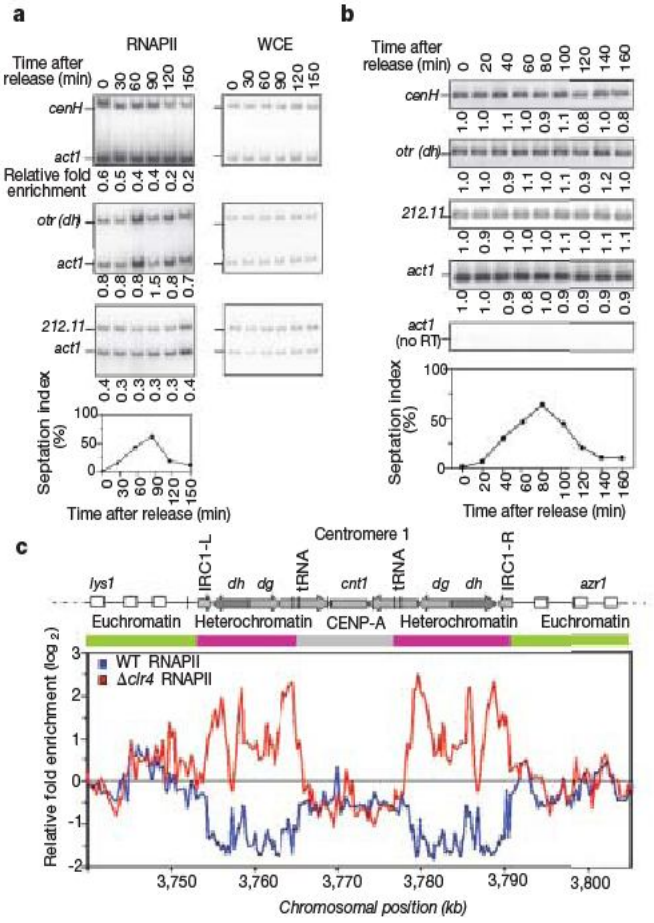


**Figure 2 | Heterochromatin limits RNAPII occupancy.** **a**, Enhanced binding of RNAPII to repeats throughout the cell cycle in *Δclr4* cells. Localization of Rpb1 at the aforementioned heterochromatic loci, relative to that of *act1*, was assayed in *Δclr4* cells. The enrichment values are shown below each lane. **b**, Heterochromatic repeats are constitutively transcribed in *Δclr4* cells. RNA samples prepared from synchronized *cdc25-22 Δclr4* cells were used to perform RT–PCR. Septation index was used to monitor cell cycle progression. **c**, Heterochromatin reduces RNAPII occupancy at the centromeric repeats. ChIP–chip was performed to measure RNAPII levels. Levels of RNAPII at *cen1* and surrounding euchromatic loci in wild type and *Δclr4* cells are plotted. IRC1-L and IRC1-R are inverted repeats flanking centromere 1 (ref. 3).

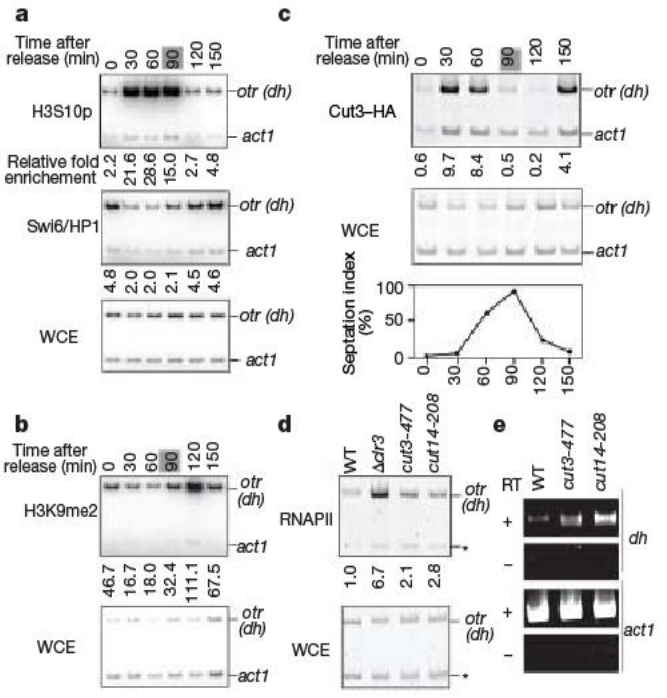


**Figure 3 | Cell-cycle-dependent changes in heterochromatin.** H3S10ph correlates with a decrease in heterochromatin levels at centromeric repeats. **a**, **b**, Levels of H3S10ph (top), Swi6 (bottom) (**a**) and H3K9me2 (**b**) at the *dh* repeats were assessed by ChIP. Grey shading indicates the peak septation index time point (90 min). **c**, Condensin recruitment to centromeric repeats coincides with a decrease in Swi6 levels during mitosis. Localization of Cut3–HA at *dh* was analysed by ChIP. **d**, Condensin mutants affect RNAPII occupancy at centromeric repeats. ChIP analyses of RNAPII (Rpb1) at the centromeric sequences in wild type (WT), *Δclr3*, *cut3-477* and *cut14-208* mutant cells at 26 °C. Asterisk indicates control band corresponding to a non-transcribed region. **e**, RT–PCR analysis showed increased accumulation of *dh* transcripts in condensin mutants. –RT, no reverse transcription. *act1* transcripts were assayed as an amplification control.

that H3S10ph regulates chromosome condensation by facilitating condensin association with chromosomes[21].

In other organisms, condensin has important roles in maintaining both heterochromatin structural integrity and gene silencing[22,23]. We asked whether condensin regulates RNAPII occupancy at heterochromatic repeats. Indeed, the levels of RNAPII and centromeric repeat transcripts were elevated in condensin mutants (*cut3-477* and *cut14-208*)[24] compared to those in wild-type cells (Fig. 3d, e). Thus, in addition to mitotic chromosome condensation, condensin subunits also participate in silencing of heterochromatic repeats.

The loss of condensin and low Swi6 levels at heterochromatic loci during S phase might provide an opportunity for RNAPII transcription through recruitment of factors such as histone H2B ubiquitin ligase complex (HULC) that has been shown to promote RNAPII occupancy at heterochromatic repeats[25]. As predicted, the HULC subunit Shf1 bound centromeric repeats preferentially during S phase (Supplementary Fig. 5), coincident with the peaks of RNAPII at these loci (Fig. 1).

Repressive heterochromatin is restored as cells traverse S phase (Fig. 3a). We investigated whether S-phase transcription of centromeric repeats promotes loading of heterochromatin factors. Localizations of Raf2 (ref. 26)—a subunit of the Clr4 complex—and Clr4 revealed cell cycle distribution profiles similar to those of H3K9me and Swi6 (Fig. 4a; data not shown). Raf2 and Clr4 levels decreased markedly during mitosis but increased gradually around S phase. However, peak enrichments of these factors, which occurred around G2 phase, were not correlated with RNAPII localization at repeats. Notably, the binding of another Clr4 complex component, Rik1, which is critical for loading Clr4 onto chromatin[7], peaked during S phase, coincident with the peak of RNAPII at the repeat loci (Fig. 4a). The preferential loading of Rik1 during S phase was restricted to transcribed centromeric repeats and was not observed at a transcriptionally inert region within a heterochromatic domain (Supplementary Fig. 6). Thus, RNAPII transcription might facilitate Rik1 recruitment to centromeric repeats during S phase. Indeed, increased transcription of repeats in a histone deacetylase mutant correlated with enhanced Rik1 loading in an RNAi-dependent manner (K.Z. and S.I.S.G., unpublished data). Moreover, Ago1, the catalytic component of the RITS RNAi effector complex, reported to interact with RNAPII physically[27], was localized at *dh* repeats during S phase and persisted into G2 phase (Fig. 4b).

RNAPII has been shown to target the H3K36 methyltransferase Set2 to transcribed regions[8], which is believed to facilitate the recruitment of a histone deacetylase (HDAC) complex via the

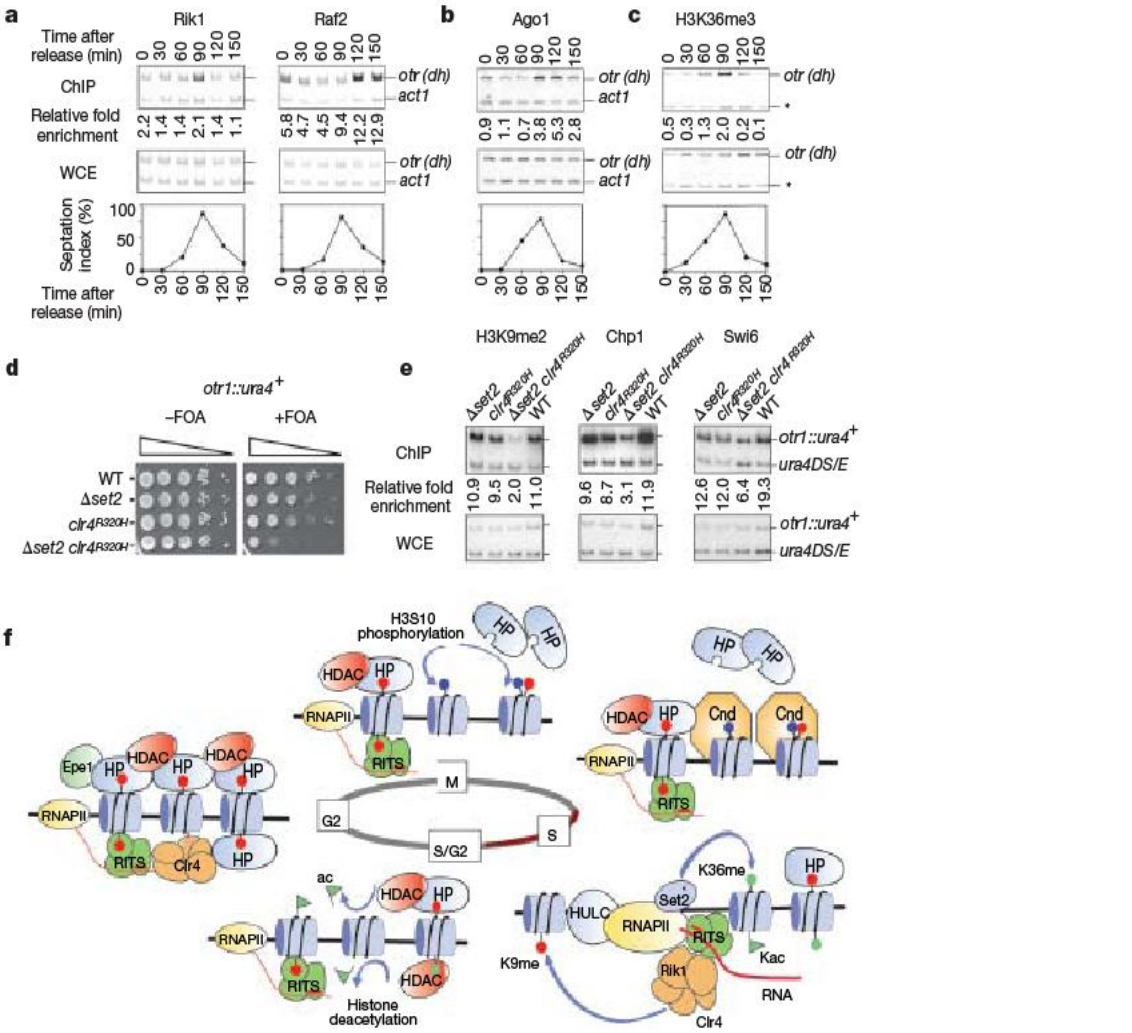


**Figure 4 | Heterochromatin assembly during S phase requires RNAPII-associated activities.** **a**, **b**, Cell cycle ChIP analyses of Clr4 and RITS complex subunits. Localizations of Rik1, Raf2 and Ago1 at *dh* were assessed by ChIP. **c**, H3K36me3 is enriched at *dh* repeats during S phase, as assessed by ChIPs. Asterisk indicates a control band corresponding to a non-transcribed region. **d**, Set2 acts in conjunction with Clr4 to maintain heterochromatic silencing. Serial dilution of wild type and mutant strains were spotted onto the indicated media to assay *otr1::ura4*$^{+}$ expression. FOA, 5-fluoroacetic acid. **e**, Loss of Set2, in the *clr4*$^{R320H}$ background, disrupts heterochromatin integrity. Levels of H3K9me2 (left), Chp1 (centre) and Swi6 (right) at *otr1::ura4*$^{+}$ were determined by ChIP. **f**, Model showing heterochromatin dynamics during the cell cycle. During G2, heterochromatin proteins (HPs) Swi6 and Chp2 bound to H3K9me not only recruit silencing factors (HDACs) but also anti-silencing factors (Epe1). H3S10ph coincides with the decrease in Swi6 levels and recruitment of condensin (Cnd, brown hexagon). HULC might facilitate RNAPII transcription during S phase. RNAPII targets H3K36me by Set2 but might also recruit Clr4, presumably through Rik1, to methylate H3K9, and RITS via Ago1 for siRNA production. H3K36me and H3K9me directly or indirectly (via HPs) target HDACs to restore G2 heterochromatin.

chromodomain of the Alp13/Eaf3 proteins[12,28]. We found that H3K36me and Alp13 were preferentially enriched at heterochromatic repeats during S phase (Fig. 4c and Supplementary Figs 7 and 8), coincident with RNAPII transcription. Moreover, our analyses indicate that Set2 and Alp13 act in a pathway parallel to Clr4 to promote heterochromatic silencing. Deletions of *set2* or *alp13* alone had little effect on expression of $ura4^+$ inserted at centromeric repeats ($otr1::ura4^+$). However, combining either of these mutant alleles with a partial loss-of-function allele of *clr4* ($clr4^{R320H}$)[13] resulted in cumulative defects in heterochromatic silencing that correlated with defects in targeting of H3K9me and chromodomain proteins Swi6 and Chp1 (a RITS subunit) (Fig. 4d, e and Supplementary Fig. 8). Loss of Set2 and Alp13 was also found to affect the expression of the forward strand of the *dh* repeat in a similar manner (Supplementary Fig. 9)[12].

This study provides insights into a sequential series of events occurring at heterochromatic repeats during cell cycle progression (Fig. 4f). S-phase transcription of the forward strand of centromeric repeats might generate short interfering RNAs (siRNAs). However, siRNA levels, although detected throughout the cell cycle, increased slightly during S/G2 phase (Supplementary Fig. 10), concomitant with elevated levels of chromatin-bound RITS required for efficient processing of nascent repeat transcripts[1]. It is possible that low-level reverse transcripts are sufficient to maintain a pool of siRNAs, whereas forward strand transcripts, which may represent spurious transcription initiating at cryptic promoters[12], instead induce heterochromatin targeting by serving as a docking platform[29] for RNAi-dependent loading of silencing factors. Indeed, the loading of Rik1 to heterochromatic repeats is coupled to RNAPII transcription. RNAPII also targets H3K36me by Set2, which in turn probably facilitates HDAC recruitment. Together with previous observations[1], these analyses suggest dual modes for targeting heterochromatin and RNAi components to centromeric repeats: via a process coupled to RNAPII transcription of repeats or by chromodomain proteins (such as Swi6, Chp1 and Chp2) bound to H3K9me (Fig. 4f). Distinct modes of heterochromatin targeting during different cell cycle phases, as well as repressive effects of condensin, probably ensure efficient silencing of centromeric repeats for most of the cell cycle. Moreover, the cyclic series of events that couple RNAPII transcription of heterochromatic sequences to DNA replication might facilitate stable propagation of heterochromatic structures through successive rounds of cell division. In this scenario, chromatin assembly factors associated with RNAPII, replication factors and/or Swi6/HP1 could promote inheritance of the heterochromatic epigenetic state[18,30].

## METHODS SUMMARY

Constructions of *Δclr4* and $clr4^{R320H}$ alleles and their effects on H3K9 methylation at heterochromatic loci were described previously[3,13]. Standard methods were used to construct strains carrying deletion alleles or epitope-tagged proteins. Cell synchronization of *cdc25-22* temperature-sensitive mutant strains was performed as described previously[10]. ChIP and ChIP–chip analyses were performed as described previously[3]. Antibodies used were anti-Rpb1 (8WG16, Covance), anti-Flag (M2)-conjugated agarose (Sigma), anti-HA (Roche), anti-Chp1 (Abcam), anti-Swi6[18], anti-H3K9me (Upstate), H3K36me[8] and anti-H3S10ph[15,17]. For RT–PCR analyses, total RNA extracted from cells was treated with RQ1 RNase-free DNase (Promega). One-hundred nanograms of RNA was amplified using the Onestep RT–PCR kit (Qiagen).

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**Supplementary Information** is linked to the online version of the paper at www.nature.com/nature.

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# Supplier **Showcase**

# The Tissue Issue

## Kits and systems for cell and tissue culture

Cell and tissue culture are topical. As was recently reported in *Nature Medicine, doi:10.1038/nm1684*, researchers at University of Minnesota injected cells from newborn rats into a thoroughly cleaned dead rat heart, and ultimately succeeded in producing a pulse. Cell and tissue culture represent new frontiers in science. And the industry suppliers here are provisioning the journey.


Image supplied by Beckman Coulter

**Beckman Coulter** introduces two new **IOTest reagents – Anti-NTB-A-PE** and **CD226 (DNAM-1)-PE**. Both are monoclonal antibodies (mAbs) to cell surface receptors for NK (natural killer) cell studies, which provide important information in disease research. These new flow cytometry reagents are compatible with Beckman Coulter's FC 500 series and Cell Lab Quanta SC systems as well as other instrument platforms. NK cells are known to discriminate between normal and virus-infected or tumor cells and they express different types of cell surface functional receptors. Inhibitory NK receptors detect surface molecules specifically expressed on normal cells and they transmit signals that spare these cells from attack. Activating NK receptors detect surface molecules that are upregulated in virus-infected and tumor cells but powerless in normal cells. The IOTest Anti-NTB-A-PE reacts with a member of a newly discovered family of NK receptors called SLAM (signaling lymphocyte activation molecule), which are important functional receptors in lymphocytes and other leukocytes. In certain cases of NTB-A expression, downstream mechanisms are thought to be involved in induction of T cell costimulation and in regulation of B cell tolerance. IOTest CD226 (DNAM-1)-PE recognizes a putative cell-corruption surveillance receptor in NK and cytotoxic T lymphocyte cells. It may also play a role in transendothelial cell migration. These two new antibodies complete Beckman Coulter's NK-targeted mAb family. The preformulated, standardized IOTest Anti-NTB-A-PE and CD226 (DNAM-1)-PE antibodies are provided in 50-test vials and are labeled for research use only.

**Sarstedt** announces several improvements to its line of **vacuum unit** and **bottle top filters**. All filters use a low binding, fast throughput PES membrane with a pore size of either 0.22µm or 0.45µm. Lot number, membrane material, and pore size are conveniently imprinted onto each filter housing. The redesigned ported neck is compatible with 45mm receiving bottles and is securely attached to the filter housing for an improved connection. Vacuum units are assembled with new ergonomic receiving bottles that feature a wide base for stability and two opposing indented sections for improved grip and handling. Sarstedt vacuum units, bottle top filters, and receiving bottles are available in 250ml and 500ml volume options. All items are individually wrapped, sterile, and certified non-pyrogenic and non-cytotoxic.

**Thermo Fisher Scientific** introduces its new **Thermo Scientific AquaTec water preservation cell**, for the effective prevention of waterborne contamination in $CO_2$ incubators and water baths. Designed to provide worry-free sample incubation and cell culture, the AquaTec provides up to six months of protection from more than 600 types of bacteria, viruses, molds and fungi. The Thermo Scientific AquaTec enables the prevention of microbes from water without the use of harsh chemicals, making it safe to handle and requiring no special disposal protocols. The AquaTec is designed and tested for all types of laboratory water and is suitable for use in equipment from any manufacturer. The Thermo Scientific AquaTec is applicable across a broad range of biological research temperature environments. As a cost-effective tool, just one three-inch AquaTec cell placed into the water reservoir provides long-lasting disinfection without the need for mixing or measuring potentially hazardous materials. Self regulation maintains the correct level of anti-microbial concentration regardless of

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BINDER introduces the C 150 $CO_2$-incubator.


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water level, negating the need for constant monitoring and testing, or equipment dismantling, and saving valuable research time. Easy to use, the AquaTec has optional suction cups for placement and fixation in the water bath or incubator reservoir, and a convenient peel-off reminder calendar that indicates when replacement is recommended.

The new **BINDER C 150 $CO_2$-incubator** provides high temperature precision with an interior that is condensation-free even at high humidity levels and a FPI infrared measuring system without drift error to ensure stable pH values with optimal cell cultivation. The seamless, deep-drawn inner chamber has 27% less surface area than the previous version, and thus less potential surface for contamination. In laboratories, it can be easily stacked without sacrificing any ease of use. Interior fittings have been reduced to a minimum for a great usable-space-to-volume ratio. The optimal incubator for all routine applications, including incubation of monolayer cultures for different cell lines, cell culture technologies, and cell-based assays in cell biology used for basic research at universities and research institutions.

**Aurelium's EpitexTM FFPE tissue kit** is a detergent-free procedure intended to extract full-length proteins and short polypeptides from formalin-fixed paraffin-embedded tissue slides. This extraction buffer allows best preservation of protein epitopes for antibody recognition. Following extraction, samples can be utilized for downstream applications such as SDS-PAGE and western blot analyses and immunoassay procedures (ELISA), in addition to other biochemical analysis and proteomics biomarker discovery projects. Advantages include highly preserved epitopes for antibody recognition, optimized extraction buffer compatible with high throughput biomarker discovery programs, full-length proteins and short polypeptides-suitable for western blotting and other immunoassays, minimal sample requirements of 1 or 2 100mm² sections, and detergent free extractions for proteomics methods.

**QIAGEN** launches a new product that supports the genomic analysis of fixed tissue samples. The **REPLI-g FFPE Kit** eliminates problems associated with DNA fragmentation and damage caused by formalin fixation. It uses a novel DNA processing reaction, allowing the advantages of multiple displacement amplification (MDA), a key technology in the amplification of genomic DNA, to be extended to highly degraded DNA samples derived from FFPE tissue. Isolation of sufficient genomic DNA from formalin-fixed, paraffin-embedded (FFPE) tissue is often difficult due to the low amount of DNA available. The REPLI-g FFPE Kit allows amplification of precious sample material while maintaining locus representation, enabling unlimited downstream analyses to be performed. Highly uniform whole genome amplification directly from formalin-fixed, paraffin-embedded (FFPE) tissue can now be performed without the need for prior DNA isolation. The REPLI-g FFPE Kit provides uniform amplification resulting in scalable and standardized DNA yields. Following lysis of the tissue section, the DNA is processed using novel buffers and enzymes that ligate fragmented DNA. The long DNA strands created by the ligation reaction are amplified using proven REPLI-g technology. A two-hour amplification reaction typically yields 10µg DNA, whereas 40µg can be obtained after eight hours incubation. Once amplified, the DNA is suitable for immediate use in most downstream genotyping assays without further purification.

The new **CDM HD**, from **FiberCell Systems**, is a chemically defined, protein free serum replacement that permits any basal medium such as DMEM or RPMI to be used without serum. CDM HD is designed for the culture of cells at high density that can permit growth in a simplified medium. Secreted products such as monoclonal antibodies and recombinant proteins are free of contaminating proteins from the medium and can be purified using simpler protocols, increasing net yield in many cases. CDM HD provides lot-to-lot consistency and is an economical replacement for serum. It is available as a dry powder to make up one liter and is used at a concentration of 10%.

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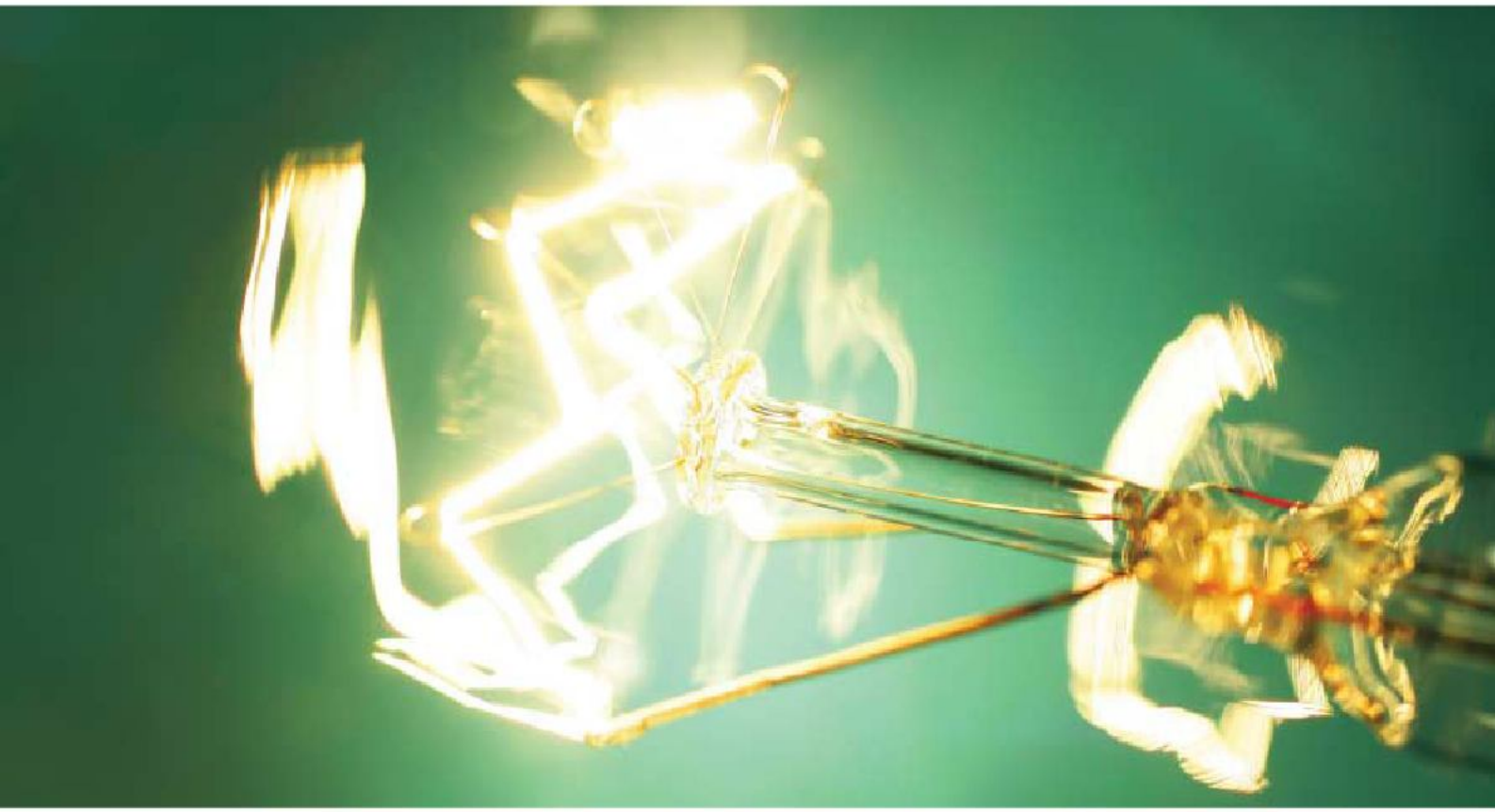

# Brilliant perspectives

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# naturejobs

This year's list of the top 100 places to work in the United States as decided by *Fortune* magazine contains only two life-sciences companies. Biotechnology firm Genentech of South San Francisco made the top 10 with a ranking of number 5, and the US arm of pharmaceutical giant AstraZeneca in Wilmington, Delaware, came in at number 83. This is something of a decline for such companies — the previous two years saw four life-sciences firms, among them Genzyme of Cambridge, Massachusetts, and Amgen of Thousand Oaks, California, grace the list.

A place in this listing is secured only after a long and involved vetting process. Four hundred employees at AstraZeneca, for example, were surveyed on issues such as how much they valued their work, camaraderie in the workplace and leadership in the company. The firm also had to describe in detail its employee benefits — such as educational opportunities, training, childcare facilities and pay — as well as supplying essays written by various people from within the company. Andrea Moselle, a senior manager in AstraZeneca's personnel department, attributes the *Fortune* listing to factors such as the firm's child and disability care, and programmes such as 'College Coach', which advises parents on how to help their children with college applications and getting financial aid.

But it is the miscellaneous perks that make some of the companies listed stand out. Genentech gives a daily subsidy of $4 to employees who come to work by bike, on foot, on public transport or who use the carpool. Google, which received the number 1 ranking for the second year running, gives employees $1,000 towards the purchase of a hybrid or electric car, and has a discount programme for California employees who install solar panels in their homes. But perhaps most impressive is Chesapeake Energy of Oklahoma City, a natural-gas producer that is number 61 on the list. It pays for its employees to earn their scuba-diving certification in the firm's own Olympic-sized pool. Maybe life-sciences companies should try investing in wet suits and oxygen tanks?

**Gene Russo, acting editor of *Naturejobs***

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## JOBS OF THE WEEK

**Postdoctoral Research programs**
National Research Council
Washington, D.C. (USA)
**Turn to page 5**

**Parasitology / Avian Immunology / Bee or Insect Immunology / Biochemistry / Virology (m/f)**
The Fraunhofer Institute for Cell Therapy and Immunology
Leipzig (Germany)
**Turn to page 9**

**Various Postdoctoral**
Pfizer
Sandwich (UK)
**Turn to page 1**

**Postdoctoral Fellowships**
Canadian Blood Services
Ottawa, Ontario (Canada)
**Turn to page 8**

**Electron Microscopy Manager**
University of Cambridge
Cambridge (UK)
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# READY, SET, HIRE

For junior science-faculty members and staff, hiring researchers is an important way to boost career success. But without management training, it's a shot in the dark. **Genevive Bjorn** reports.

EYEWIRE

When systems neuroscientist Bijan Pesaran landed his first faculty job at New York University in 2005, he needed to hire a research team. Although he was lucky enough to find a postdoc straight away, hiring other team members seemed to be an overwhelming task. He turned to his senior colleagues for advice, which helped — but it wasn't enough. Hiring was the thing he knew least about. So Pesaran took a scientific management training class offered through the Howard Hughes Medical Institute (HHMI; see 'Making the right moves').

The class soon paid off. To find a technician, Pesaran advertised through an Internet job site, received heaps of mostly unsuitable responses and interviewed many candidates, eventually finding one whose enthusiasm outweighed her relative lack of experience. The management training course had prepared Pesaran to carry out the essential tasks of hiring and team building. Indeed, two years later, he has six people working in a productive lab and no regrets.

Making a productivity-boosting appointment is not a formal part of PhD curricula or the research culture, but poor or delayed hiring decisions can strain a young career. Navigating recruitment issues — such as how to go about finding a suitable postdoc or technician, honing your interview techniques, negotiating salary and motivating people to get the best results — can seem daunting. For many, it's a huge shock for which they are unprepared.

## What's not taught

"Most postdocs don't get training in management skills," says Alyson Reed, executive director of the US National Postdoctoral Association. "But being able to put together and manage a team becomes a vital part of every scientist's career."

Management training could easily be built into career paths, perhaps in the form of mentoring or seminars tailored specifically to the needs of young scientists, says Janet Metcalfe, director of the UK GRAD Programme, an organization that provides support to postgraduate researchers. Part of it would involve making trainees more aware of the non-scientific skills they use regularly, such as communication, organization and leadership. The first step is getting young scientists to recognize that these skills are as important as their scientific ones. "Researchers often find it surprisingly difficult to reflect on their competency in areas other than their science," says Metcalfe.

**"It is very interesting to see what people are saying about you. I took those lessons to heart."**
— Bijan Pesaran

Learning about his non-scientific skills was a key part of Pesaran's HHMI training. He benefited from taking a personality profile and receiving anonymous feedback. "It is very interesting to see what people think about you," says Pesaran. "I took those lessons to heart." He has tried to improve his patience and tolerance as a result of the comments.

Several institutions in the United States and Europe, such as the European Molecular Biology Organization (EMBO), offer seminars based on the HHMI model for either science postdocs or junior science-faculty members. Cassandra Extavour took EMBO's lab management course in 2006 while doing a postdoc at the University of Cambridge, UK — before she started her faculty job at Harvard in evolutionary biology.

Compared with colleagues who don't have this kind of training, Extavour says that she is less stressed as she isn't starting from scratch. Although postdocs can focus on their research, junior faculty members also have to prioritize and juggle hundreds of tasks they know nothing about — including hiring. "The EMBO course presented some useful ideas on how to decide what's important as well as very practical advice on interviewing, hiring, team building, coaching, mentoring and conflict management," says Extavour. "I wouldn't have got this training from my job."

Javier Martinez, junior group leader at the Institute of Molecular Biotechnology of the Austrian Academy of Sciences in Vienna, took EMBO's training course in 2004. He describes it as intensive but helpful in dealing

with issues that come up every day for scientists, including making that crucial first postdoc hire.

"Your postdoc is the person who will help you train other PhD students and will be the experienced one pipetting by your side," says Martinez. "You have to remember that you were a postdoc not so long ago." The skills he learned in the management course prepared him to make the transition from postdoc to group leader himself and to choose the right person.

Learning what hiring challenges to expect and how to deal with them is an important part of these training programmes. One common challenge faced by junior faculty members is the urge to make a decision on the basis of immediate research needs, rather than what might be needed in the next three to five years.

"It's important to think long term," says Extavour. Another challenge is feeling lonely in a new job and approaching the interviews with candidates as if making a new friend, she warns. "It's important not to forget why you are on the hiring side of the table," she says.

Learning a few hiring strategies is another important part of these training programmes. Writing a thorough job description before posting help-wanted ads can make the whole process more efficient. Those hiring should include any must-have qualifications, such as an academic degree; other highly desirable skills, such as experience with animals or programming; and optional skills, such as experience with certain types of reporting or writing. Being as specific as possible leads to better candidates, a faster screening process and more discerning interview questions later.

Another strategy is to compare apples with apples by asking each prospective candidate the same interview questions. It's also important to avoid asking personal questions about marital and family status, which are potentially discriminatory and illegal. And interviewers should watch for any red flags that may come up, such as lack of enthusiasm for the job, complaints about previous advisers or colleagues, or simply avoiding questions.

Once the recruitment, screening and interviews are complete, a helpful strategy for evaluating candidates is to assign each one a numerical grade immediately after the interview. At the end of the process, compare the pool and make a shortlist of the best three or four. Make an offer to the top candidate as early as possible, and let the others know that they are on the shortlist. Be prepared to wait for them to choose among other offers. Top candidates will be in demand. It may be necessary to offer enticements, such as a new computer and paying for publications, in order to get the best candidate.

P. FETTERS/HHMI

**Courses in scientific management can be invaluable for junior faculty members.**

## MAKING THE RIGHT MOVES

In 2002, the Howard Hughes Medical Institute (HHMI) and the Burroughs Wellcome Fund recognized a pressing need for additional career training. So they offered a lab-management workshop to members of their research community, which included junior faculty members and recipients of funding awards.

"The participants raved how useful it was and wanted the programme to become widely available," says Maryrose Franko, senior programme officer at the HHMI.

Instead of publishing the results as proceedings, the workshop was morphed into a freely available book called *Making the Right Moves* and a training course called 'Training Scientists to Make the Right Moves'.

One book chapter, for example, breaks the hiring process down into easily digestible pieces that include how to recruit, screen and evaluate applicants. It also has tips on interview questions and techniques.

The programme has expanded to become a model for scientific management training and 72 institutional departments have requested copies of the second edition of the book. **G.B.**

### More responsibility earlier

Not everyone waits to reach postdoc or junior faculty stage before learning essential management skills. Globally, fewer than 30% of PhD scientists go on to work in academia, which means that most researchers are looking for jobs in industry or government. Those jobs often require some management know-how.

Even without formal training, there are practical ways to go about gaining some management skills. Koen van Dam, a PhD candidate at Delft University of Technology and president of Eurodoc, the European council of doctoral candidates and young researchers, worked at developing his own relevant skills set. He helped to interview and evaluate some PhD student candidates, which gave him an insight into the hiring process.

This kind of initiative gives young scientists a sense of the nuts and bolts of hiring. "Even if you've received some scientific management training it is very useful to have a working general knowledge of local labour laws and hiring practices," says Extavour. She also recommends finding out as early as possible about the department's specific recruitment practices, because extra layers of paperwork could add more time to the hiring process.

Figuring out how to make a career-boosting appointment at the junior faculty or staff phase requires a self-awareness that less-stressed researchers seem to grasp: acknowledging that scientific management skills are needed, knowing where to look for help and mustering the resources to go and do it. The long-term career pay-off of making a successful first hire is potentially huge — whether it's winning a future tenure bid or landing a dream job outside academia.

And scientific management training is a key component in the drive for success. "Postdocs are excited about it. Junior faculty are desperate for it. But senior faculty still tend not to see the point," says Extavour. ■

**Genevive Bjorn is a freelance writer in Maui, Hawaii.**

**Correction**

In the Regions story 'Argentina's pivotal moment' (*Nature* **451,** 494–496; 2008) the picture caption on page 496 transposed the names of the two people. Marcelo Rubinstein was pictured top, and Martin Giurfa was below.

# MOVERS

**Eva Feldman, director, Taubman Medical Research Institute, University of Michigan Medical School, Ann Arbor, Michigan**

**2005–present:** Director, Neuropathy Center, University of Michigan, Ann Arbor, Michigan
**2001–present:** Director, ALS clinic, University of Michigan
**2000–present:** Professor, Department of Neurology, and Director, Juvenile Diabetes Research Foundation Center, University of Michigan

**Eva Feldman's affable demeanour belies a tenacity that has enabled a successful and wide-ranging career in neuroscience. At the age of 11, she took solo bus rides to downtown Indianapolis to volunteer at a hospital as a way to explore her medical aspirations. Undeterred by repeated advice that she pursue a less demanding career, she became an academic clinician. In her new role as director of the Taubman Medical Institute at the University of Michigan, she wants to promote high-risk, high-reward research.**

**After studying biology and chemistry, Feldman decided to pursue a master's degree in neuroscience at Indiana's University of Notre Dame. But while pursuing her MD-PhD at the University of Michigan she found that she loved being a clinician. She became chief resident at Johns Hopkins Hospital in Baltimore, Maryland, and the first neurologist to win its medical teaching award.**

**She returned to the University of Michigan to begin her career, in part because her husband accepted a job in the area. Setting aside institutional 'in-breeding' concerns, she says her familiarity with the place has helped her fully realize research opportunities.**

**For example, as part of a programme in neurology research and discovery started in 2000, she was able to build multiple collaborations across departments. Since then she has created, and become director of, two centres and a clinic focused on the complications of diabetes, amyotrophic lateral sclerosis (ALS, or Lou Gehrig's disease) and, most recently, neuropathy. She runs a neuroscience lab of 14 postdocs and nine research assistants.**

**The secret of her success, she says, is delegation. She credits management courses on executive leadership in academic medicine with teaching her to create an infrastructure to juggle the needs of so many posts — even using technology such as wikis to post project materials.**

**As director of the new Taubman Medical Research Institute, she plans to oversee novel high-risk research — not typically funded in today's competitive climate — including the pursuit of stem-cell-based ALS therapies. Her colleagues are excited to have a leader eager to take risks at a time when most academics put forth conservative proposals to secure funding from the National Institutes of Health.**

**"The successes may not outnumber the failures, but new, innovative treatments will be worth the risk," says Sid Gilman, director of the University of Michigan's Alzheimer's Disease Research Center.** ■

**Virginia Gewin**

# NETWORKS & SUPPORT

## Japanese postdocs seek their path

Responding to concerns over the uncertain career paths for postdocs in Japan, we recently carried out a survey at seven universities — Osaka, Tohoku, Hokkaido, Waseda, Nagoya, Yamaguchi and Kyushu — and the institute of physical and chemical research (RIKEN). Our fields included science, engineering, agriculture and health care. It was the first such survey at multiple institutions in Japan.

A total of 3,870 people responded just after the end of fiscal year 2005: about a quarter of the roughly 15,000 postdocs in Japan during that period. Two-thirds (2,592) stayed postdocs at the same institution they'd been in during the year; 8% (310) had become postdocs at other institutions; 19% (752) were doing other work, studying or unemployed; and the occupations of the remaining 6% (216) were unknown. Of the 752 who changed their type of work or role, 82% entered research and development (R&D) professions and 9% entered occupations requiring specialized knowledge (such as teachers, doctors, occupations related to intellectual property, coordinators for industry–university collaboration, or science and technology communicators).

The percentage of Japanese versus foreigners who became postdocs at other institutions — as well as who became non-postdoc R&D workers — were similar. Of Japanese postdocs, 72% stayed in Japan and 7% went to the United States. Among non-Japanese, 24% stayed in Japan and 20% moved to China.

More women were unemployed at the end of 2005 than men. More engineering postdocs than scientist postdocs became R&D professionals outside Japan; in Japan, more engineers than other postdocs became private-sector R&D personnel. The average age of becoming a lecturer was 34.2, associate professor 36.9, professor 44.4 years. The number of people becoming postdocs at other institutions decreased with age.

As this was the first large survey of its kind, it is not clear whether job prospects are getting better or worse. But it is worth noting that more than 80% of those who obtained non-postdoctoral positions were able to enter R&D professions. For each institution, the results suggest that the different forms of career development or support may be necessary, depending on the field, to diversify postdocs' career options. ■

**Toshiyuki Misu is a senior research fellow, and Akira Horoiwa an affiliated fellow, at the National Institute of Science and Technology Policy in Tokyo.**

**POSTDOC JOURNAL**

## Fruit medley

I'm a fan of quality produce. So when I told my wife that oranges and other locally grown citrus fruits were reasons for staying in Israel to pursue a faculty position, she retorted: "Get your priorities straight!"

The truth is, I'm not that shallow. I moved to Israel to study genes controlling natural variation in tomatoes, and I thought we might stay for scientific and personal reasons. But now I realize I'm as American as the New England apple pie I grew up with. So when I recently committed to job hunt in both countries, I mulled over what might become a near literal 'apples versus oranges' decision.

How should I choose? The considerations are endless: institution, colleagues, funding, a partner's career, family and friends, children's education, and the political and social climate. And then there is the need to learn skills beyond the bench, such as becoming an effective teacher, marketer and collaborator.

How am I coping? I'm gathering as much information as possible. The key to making informed decisions is seeking advice from the right sources. Consulting former mentors and colleagues who also struggled through this transition is helping to lift the fog. Merging the best of both worlds is impossible. So now I'm stressing about the decision, and when I stress, I get hungry. A fruit salad of, say, apples and oranges would hit the spot. ■

**Zachary Lippman is a postdoctoral fellow at the Hebrew University of Jerusalem's faculty of agriculture.**

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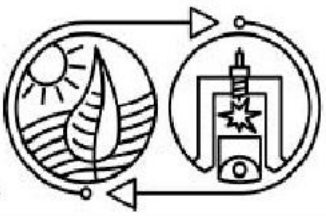

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Vetenskapsrådet

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Contact for inquiries: **wels@em.uni-frankfurt.de** or **grez@em.uni-frankfurt.de**

Please direct applications including CV, university entrance qualification (Abiturzeugnis), university certificates, list of publications, brief summary of previous research experience and two letters of reference to:

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Queta Bond
President

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Max Planck Institute for Brain Research
Prof. Dr. Dr. Wolf Singer
Deutschordenstr. 46, 60528 Frankfurt am Main/German
E-mail: singer@mpih-frankfurt.mpg.de

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## POST DOCTORAL FELLOWSHIPS

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Applications for these roles must now be made online at **http://jobs.mrc.ac.uk** If you do not have internet access or you experience technical difficulties please call 01793 301157.

The closing date is **6 March 2008**.

*The MRC is an Equal Opportunities Employer*

U124459R

dkfz.

The Medical Faculty Mannheim of the University of Heidelberg and the German Cancer Research Center has established the Joint Research Division Vascular Biology (for details see: www.angiolab.de).

*Applications are now invited for*

## Postdoctoral Positions – Angiogenesis

**Ref-No. 18/2008**

in the fields of Angiopoietin/Tie biology (VB1), tumor progression and metastasis (VB2), vascular guidance molecules (VB3) and lymphangiogenesis (VB4) in the laboratory of Prof. Hellmut Augustin.

All positions require a solid background in cell/molecular biology and/or tumor biology. Experience in vascular biology is desirable.

DKFZ wish to increase the proportion of female scientists and strongly encourage applications of qualified women. With equal qualifications, handicapped individuals will be considered preferentially.

**Please submit your application to Deutsches Krebsforschungszentrum, Personal-abteilung, INF 280, D-69120 Heidelberg, Germany (or via E-Mail personalabteilung@dkfz.de). Informal inquiries should be addressed to Prof. Hellmut Augustin (augustin@angiogenese.de or +49-6221-421500).**

W124371R

MRC Medical Research Council

MRC Laboratory of Molecular Biology, Cambridge

## Postdoctoral Position

### £25,368 - £26,953 per annum

Applications are invited for a postdoctoral position in the group of Dr. Phil Holliger. Our research focuses on the synthetic biology of nucleic acid replication (see Nature Biotechnol. (2004), 22, 755 & Nature Biotechnol. (2007), 25, 939). The aim of the project is to apply compartmentalisation and selection strategies developed in the laboratory to the evolution of novel DNA polymerases. We are particularly interested in developing an artificial genetic system and study its functionality with respect to information transfer, heredity and evolution. You must have a PhD degree, and experience in recombinant DNA technology.

You will be awarded an MRC Career Development Fellowship, which is a three year training and development position for a post-doctoral scientist who has recently completed their doctoral studies or is moving into a new research discipline. For further information about the Laboratory of Molecular Biology, please visit www.mrc-lmb.cam.ac.uk
For further information about this position, please contact Dr. Phil Holliger, email: ph1@mrc-lmb.cam.ac.uk

The salary is supported by a flexible pay award policy, 6 weeks annual leave and public holidays, optional MRC final salary pension scheme and excellent onsite sports and social facilities.

This position is subject to pre-employment screening.

Applications for this post must be made online at http://jobs.mrc.ac.uk inputting reference number: LMB08/071. If you do not have access to the internet or experience technical difficulties please contact 01793 301280.

**Closing date: 7 March 2008.**

**For further information about the MRC visit www.mrc.ac.uk**

The Medical Research Council is an Equal Opportunities Employer

'Leading science for better health'

U124494R

**www.cam.ac.uk/jobs/**
**A world of opportunities**

UNIVERSITY OF CAMBRIDGE

## Research Associate

Department of Physics
£25,134-£32,796 pa
Limit of tenure: 31 December 2010.

Applications are invited for the post of postdoctoral Research Associate to work on an EU-funded project (GRAND). The project aim is to investigate graphene as a possible material to replace CMOS devices in the 5 nm range. This post will use a low temperature scanning probe instrument to investigate how the device uniformity of narrow graphene structures is affected by functionalizing the grapheme edges.

Candidates should have a PhD in either Physics or Electrical Engineering. Experience working with processing and measuring carbon-based devices is essential as well as experience with measurements performed at cryogenic temperatures. Experience with atomic force microscopy is also required.

**For further details of the post, please visit our website http://www.sp.phy.cam.ac.uk**

**Completed applications, including a Curriculum Vitae, names of two referees and a PD18 cover sheet (parts I and III only and available from www.admin.cam.ac.uk/offices/personnel/forms/pd18/), should be addressed to: The Group Administrator, Semiconductor Physics Group, Cavendish Laboratory, JJ Thomson Avenue, Cambridge CB3 0HE, or e-mail to: admin@sp.phy.cam.ac.uk.**

**Quote Reference: KA02972.**

**Closing Date: 1 April 2008.**

**The University is committed to Equality of Opportunity.**

U124455R

## The Future Begins with Us.

RWTH AACHEN UNIVERSITY

With 30,000 students and 10,000 employees, RWTH Aachen University is one of the leading universities and also the largest employer and training body in the region. Its teaching and research are closely connected to industry and are characterized by an international, innovative, and interdisciplinary approach.

## Postdoctoral positions in catalysis research

**Institute for Technical and Macromolecular Chemistry (ITMC)**

**Our profile**
We invite highly motivated young scientists to apply for the available postdoctoral positions at the new centre of catalysis research, which was recently established at the Institute for Technical and Macromolecular Chemistry (ITMC), RWTH Aachen.

**Your profile**
Applications of outstanding researchers with recognized accomplishments in any field of chemistry and chemical engineering will be considered. Experience in polymer chemistry (coordinative polymerization), synthetic organic chemistry, reaction engineering, organometallic chemistry, and/or homogeneous and heterogeneous catalysis, is preferred, but not required.

**Your duties and responsibilities**
The research at the centre is focused on fundamental research in the fields of homogeneous and heterogeneous catalysis with special emphasis on the following topics:

- Synthesis of low molecular weight building blocks for polymers
- CO2 fixation in polymers
- Activation of CH bonds in hydrocarbons
- Partial oxidation reactions
- Redox catalysis.

**Our offer**
The positions are for a fixed term of 1 year with the option of renewal. Review of applicants will begin immediately and continue until positions are filled.
RWTH Aachen University has been rewarded with the "Total-E-Quality-Award" for its efforts with respect to gender equality. In cases of equal qualification, aptitude and expertise of the applicants, female applicants will be given preferential treatment for those salary groups and careers in which females are underrepresented, unless there are preponderant reasons to give preference to another applicant. Please refer to § 8 Article 6 of the North Rhine-Westphalian Equal Opportunities Act (Landesgleichstellungsgesetz NW).
RWTH Aachen University has been rewarded with the title "disability-friendly" ("Prädikat behindertenfreundlich" ) for its efforts with respect to training and employment of severely disabled people. Applications from severely disabled people with appropriate suitability are explicitly welcome. This also applies to people with equal opportunities in accordance with § 2 SGB IX (Social Code).

**Your contact person**
For advance information, please contact Dr. Thomas Müller on tel. no. +492418026497 or e-mail thomas.mueller@catalyticcenter.rwth-aachen.de You can also obtain further information from our websites: http://www.itmc.rwth-aachen.de. Please send your application to Dr. Thomas Müller, Institut für Technische und Makromolekulare Chemie, RWTH-Aachen, Worringerweg 1, 52074 Aachen.

W124531R

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*Dedicated to Discovery...Committed to Care.*

## RESEARCH FELLOW

*Mid-sized laboratory at the world-renowned Dana-Farber Cancer Institute and Harvard Medical School seeks an energetic post-doctoral fellow to work at the forefront of research on apoptosis and cancer. The Letai laboratory (http://research.dfci.harvard.edu/letai/) combines investigation into very basic mechanisms of apoptotic control with translation into clinical trials. The ideal candidate will think independently, have an excellent record of accomplishment, and possess good communication skills. Applicants should forward CV, summary of research interests and contact information for three references. Experience in mitochondrial biology, stem cell biology, FACS analysis, or proteomics a plus. Applicants with PhD and MD/PhD encouraged to apply.* **Job Req: #15617.**

### Please apply online at:

**http://www.dana-farber.org/abo/working/open/job_detail.asp?jobID=15617**

HARVARD MEDICAL SCHOOL

*The Dana-Farber Cancer Institute is an Equal Opportunity Employer.* NW122472R

**SHARE THE VISION. FIND THE CURE**

## Post-doctoral position

### - Cell trafficking in inflammatory liver disease

**This post, funded by the EU Marie Curie Programme, is available to work on a project jointly held between the University of Birmingham, UK and Biotie Therapies Corp. in Turku, Finland. The position is available from April 2008. The first 2 years will be spent in the Liver Research Group within the MRC Centre for Immune Regulation at the Institute of Biomedical Research, University of Birmingham. The Institute houses 300 scientists and has state of the art facilities. The applicant will then have the opportunity to continue the project at Biotie Therapies for a minimum of one year.**

A PhD scientist is required to work on a project to characterise the function of the novel endothelial adhesion receptor VAP-1 in hepatic inflammation. The successful applicant will work within a group studying leukocyte recruitment to the liver and will use a combination of in vitro flow-based adhesion assays and in vivo models, including intravital microscopy, to determine the role of VAP-1 in liver inflammation and to develop and test new inhibitors of VAP-1 for use in inflammatory liver disease. Skills in cell or molecular biology are essential and experience of working with animal models of inflammation is desirable.

*Applications (curriculum vitae) should be submitted to* ***Prof. David Adams, Liver Research Laboratory, Institute of Biomedical Research, University of Birmingham, Birmingham B15 2TT, UK.*** *The closing date for applications is Friday 7th March 2008.*

*Further information:* ***http://medsciences.bham.ac.uk/departments/liver/staff/adams_rg.htm*** *and* ***www.biotie.com.*** *For informal enquiries e-mail* ***info@biotie.com.***

UNIVERSITY OF BIRMINGHAM

BIOTIE THERAPIES

Biotie Therapies Corp., Tykistökatu 6, FI-20520 Turku, Finland, www.biotie.com

W124567R

### THE KAY KENDALL LEUKAEMIA FUND

## INTERMEDIATE RESEARCH FELLOWSHIP

The Kay Kendall Leukaemia Fund invites applications from outstanding biomedical scientists for a four-year intermediate research fellowship to study any aspect of leukaemia.

The research will typically address one or more questions relating to the epidemiology, aetiology, pathogenesis, diagnosis or treatment of leukaemia or a closely allied malignancy. Applicants may be of any nationality and should intend to work for at least part of the fellowship in an appropriate institution in the UK. The appointment will be made on either clinical or non-clinical payscales as appropriate, and will normally be taken up within six months of the award. The fellowship will cover the cost of salaries for four years for the fellow and may support one assistant together with costs of laboratory consumables but will not contribute to departmental overheads.

The application should be received on or before 28 April 2008 (by email as well as one hard copy). It must include a scientific proposal of up to six single-spaced pages (excluding references, costings, and CV), and a statement from the departmental chairman or laboratory director that he/she is prepared to make available all appropriate facilities if the applicant is successful.

*Further information can be obtained from the website* **www.kklf.org.uk** *or contact:*

**The Kay Kendall Leukaemia Fund,**
**Allington House (1st Floor), 150 Victoria Street,**
**London SW1E 5AE**

*Telephone:* **020 7410 0330** *E-mail:* **info@kklf.org.uk**

U124058R

### THE KAY KENDALL LEUKAEMIA FUND

## SENIOR RESEARCH FELLOWSHIP

The Kay Kendall Leukaemia Fund invites applications from outstanding biomedical scientists for a five-year senior research fellowship to study any aspect of leukaemia.

The research will typically address one or more questions relating to the epidemiology, aetiology, pathogenesis, diagnosis or treatment of leukaemia or a closely allied malignancy. Applicants may be of any nationality and should intend to work in an appropriate institution in the UK. The appointment will be made on either clinical or non-clinical payscales as appropriate, and will normally be taken up within six months of the award. The fellowship will cover the cost of salaries for five years for the fellow and may support up to two assistants together with costs of laboratory consumables but will not contribute to departmental overheads.

The application should be received on or before 28 April 2008 (by email as well as one hard copy). It must include a scientific proposal of up to ten single-spaced pages (excluding references, costings, and CV), and a statement from the departmental chairman or laboratory director that he/she is prepared to make available all appropriate facilities if the applicant is successful.

*Further information can be obtained from the website* **www.kklf.org.uk** *or contact:*

**The Kay Kendall Leukaemia Fund,**
**Allington House (1st Floor),**
**150 Victoria Street, London SW1E 5AE**

*Telephone:* **020 7410 0330** *E-mail:* **info@kklf.org.uk**

U124057R

NW123580R

Canadian Blood Services
*it's in you to give*

## CANADIAN BLOOD SERVICES

# Postdoctoral Fellowships

Canadian Blood Services (CBS) is accepting applications for Postdoctoral Fellowships (PDF) to work with our affiliated Research & Development groups across Canada. CBS has active research programs within transfusion science emphasizing platelets, stem cells, plasma proteins, infectious disease, epidemiology and clinical transfusion practice. Applicants should have a Ph.D. or M.D. degree and a strong research background. This two-year award includes a salary and research allowance, and the possibility of a one-year renewal. Candidates must select and contact a CBS affiliated scientist to serve as the Postdoctoral Fellowship supervisor. CBS also supports a Graduate Fellowship Program and a Summer Internship Program. Information, forms and a list of CBS affiliated scientists are available at **www.blood.ca,** and from the **R&D Office (elaine.konecny@blood.ca), Canadian Blood Services, Research and Development, 1800 Alta Vista Drive, Ottawa, Ontario, K1G 4J5, Canada.**

Please note that the 2008 campaign will not accept on-line applications. Candidates are encouraged to respond by hard copy.

PDF Application deadline: July 2, 2008.

## Harvard University

## Department of Chemistry & Chemical Biology

## Postdoctoral Fellowships

The Mary Fieser Postdoctoral Fellowships Program seeks to enhance diversity and excellence in the Department of Chemistry and Chemical Biology (CCB) of Harvard University by providing postdoctoral fellowship support to women and groups that are historically underrepresented in science and to others whose background, experiences and research interests will contribute to academic diversity in CCB. Promising scholars male and female who have been historically underrepresented in chemistry (including but not limited to African American, American Indian, and Hispanic/Latino) are encouraged to apply.

Up to 12 fellowships may be awarded in 2008. Fellowships will be for an initial period of one year, with potential for renewal of up to one year (a two-year maximum level of support). Stipends will be targeted to the NRSA year '0' amount and will include health insurance and other benefits. Applicants are required to have completed all PhD requirements prior to arrival. All applicants must be eligible to work in the United States before arrival.

Additional information regarding the application process and department faculty are found at **http://www.chem.harvard.edu**

NW122187R

## LOVELACE RESPIRATORY RESEARCH INSTITUTE…Giving the Gift of Breath

### Associate Research Scientist or Postdoctoral Fellow

### Job #N13607

Lovelace Respiratory Research Institute (LRRI), a non-profit biomedical research organization, is seeking a motivated individual for an Associate Research Scientist or Postdoctoral Fellow with an advanced degree in the area of cell biology or a related field, as well as experience in image analysis systems for quantification of inflammatory responses, tissue damage, and epithelial cell hyperplasia. This position funded by federal and commercial sources is for individuals interested in identifying the role of inflammatory, apoptotic, neuronal, and autophagic pathways involved in cigarette smoke-induced changes in the lung. Ongoing studies involve transgenic and knockout mice, organ and cell culture models to determine the role of certain proteins in the development of emphysema and chronic bronchitis. Experience with laser capture microdissection of cells is desired. Applicants should have a PhD, at least two (2) years of directly related experience and a strong research background in histology and preferably in molecular/cell biology. Demonstrated ability to handle fundamental scientific tasks. Published and/or presented original research.

*Please send a resume or C.V. to Job* **#N13607,**
**Human Resources Office, LRRI,**
**2425 Ridgecrest Dr. SE, Albuquerque,**
**New Mexico 87108**
*or e-mail* **HRMAIL@LRRI.ORG**
*or send via Fax to* **505-348-4976**
*Equal Opportunity/Affirmative Action Employer. M/F*

NW124421R

**MAX PLANCK INSTITUTE** FOR HUMAN COGNITIVE AND BRAIN SCIENCES LEIPZIG

The Max Planck Institute for Human Cognitive and Brain Sciences in Leipzig, Germany, Department of Cognitive Neurology (Head of Department: Prof. Dr. Arno Villringer) invites you to apply for

## PostDoctoral and PhD positions

The focus of our multi-disciplinary research group is on stroke rehabilitation in humans. We are aiming to merge stimulation protocols (e. g. TMS, tDCS), cognitive tasks and drug administration to induce neuroplasticity as observed by electrophysiological methods and functional magnetic resonance imaging (alone AND in combination, e. g. fMRI-EEG). Candidates interested in clinical neuroscience, neuroplasticity, as well as in higher cognitive function and brain imaging are invited to apply. The successful candidates should be enthusiastic and will have a strong interest in this exciting research area.

A background in EEG, TMS or fMRI acquisition and analysis techniques (SPM, FSL, Matlab, BESA etc.) would be of advantage. The Max Planck Institute in Leipzig offers an excellent multi-disciplinary and interactive research environment with access to excellent research facilities (3T and 7T research MRI scanners, MEG, EEG and TMS devices). The positions are funded for maximally 3 years from now and will be held open until suitable candidates have been found.

For further details please contact Dr. Burkhard Pleger by email bpleger@cbs.mpg.de or phone: +49 (0) 341-9940-135.

In order to increase the proportion of female staff members, female scientists are particularly encouraged. Disabled applicants are preferred if qualification is equal.

Please send your application including the name of referees by email (preferred) or post, citing the **code number "D 1/08"** to:

**Max-Planck-Institut für**
**Kognitions- und Neurowissenschaften**
**- Verwaltung -**
**Stephanstraße 1a, D-04103 Leipzig**
**www.cbs.mpg.de**

W124565R

MAX-PLANCK-GESELLSCHAFT

University of Reading

www.reading.ac.uk/jobs

# Postdoctoral Research Assistant

## School of Pharmacy

This appointment is full-time, fixed-term for 3 years and starts on 1 April 2008
Grade 6 – £25,134 to £27,466 per annum

**Applications are invited from Postdoctoral Researchers for a 3-year full-time position within a vibrant and expanding research group under the supervision of Dr Gary Stephens and Dr Cornelius Krasel.**

The project will use *in vitro* electrophysiological techniques from native neurones and transfected mammalian cells, in addition to expression of fluorophore-conjugated calcium channel subunits and associated fluorescence resonance energy transfer (FRET) microscopy.

You will have:

- a PhD in a related research area
- enthusiasm and drive
- excellent communication skills
- good presentation skills
- team-working ability
- a desire to learn
- an ability to acquire and analyse data

Informal enquiries: Dr Gary Stephens on +44 (0)118 378 6156 or email g.j.stephens@reading.ac.uk

**Closing date: 1 March 2008**

Further information and application forms are available at www.reading.ac.uk/jobs, or from:
Human Resources, University of Reading, Whiteknights, PO Box 217, Reading RG6 6AH,
Telephone +44 (0)118 378 6771 (voicemail)

**Please quote reference number RS08007**

We value a diverse workforce and welcome applications from all sections of the community

U124581R

The Queen's Anniversary Prizes 2005

IZI

**Fraunhofer** Institut Zelltherapie und Immunologie

The Department of Vaccine Development of The Fraunhofer Institute for Cell Therapy and Immunology in Leipzig/Germany, invites applications for several positions in

## Parasitology / Avian Immunology / Bee or Insect Immunology / Biochemistry / Virology (m/f)

*The Fraunhofer Institute for Cell Therapy and Immunology is one of 56 institutes of the Fraunhofer-Society. As one of the leading organizations for applied research in Europe it offers ambitious scientists challenging tasks coupled with responsibility and room for creativity.*

for projects of molecular vaccine research and development for livestock animals, commencing April 1, 2008.

Preference will be given to candidates who have an outstanding expertise in one of the disciplines named above and strong interests in both fundamental and applied molecular vaccine research. The position is limited for three years, prolongation is possible.

Applicants must have a Ph.D., and relevant post-doctoral experience with an established record of research excellence, peer-reviewed publications. In addition, candidates must be highly motivated and fluent in English. The salery will be in accordance with the German tariff regulation (TVöD-Ost).
Please apply online at:
**https://jobs.fraunhofer.de/Vacancies/16674/Description**
or send your application with all associated documents (CV, brief description of research experience and career goals and the names/email adresses of 2-3 references) with the code number IZI-2008-3 by email to:
**PD Dr. Matthias Giese, Head of Vaccine Development,**
**email: matthias.giese@izi.fraunhofer.de**
**Fraunhofer Institute for Cell Therapy und Immunology,**
**Deutscher Platz 5e, 04103 Leipzig/Germany.**
**Any questions regarding this position will be answered by**
**PD Dr. Matthias Giese, email: matthias.giese@izi.fraunhofer.de**
Information regarding the Fraunhofer Institute can be found under
**www.izi.fraunhofer.de**

W124216R



**Georgetown University Medical Center**
**Molecular Tumor Biology**
**Postdoctoral position**
NW124485RL
Study novel targets and signal transduction pathways in cancer cells and to develop therapeutic strategies in animal models. Recent Ph.D. with background and training in molecular biology and biochemistry is desirable. Please send curriculum vitae and names of three references to: Usha Kasid, Ph.D., Professor, Georgetown University Medical Center.

**Contact**

Room E208, The Research Building
3970 Reservoir Road NW
Washington DC 20057

**University of Kentucky**
**Biomedical Engineering**
**Postdoc in Bio-photonics**
NW124385RL
Responsibilities consist of design and development of diffuse optical spectroscopy/imaging systems, image reconstruction, and *in vivo* monitoring of tissue hemodynamics. Submit the CV and statement of interests to Bio-photonics Lab (http://www.cbme.uky.edu/yu.htm), Center for Biomedical Engineering, University of Kentucky (guoqiang.yu@uky.edu)

**Contact**

Guoqiang Yu Tel: 859-2579110
E-mail: Guoqiang.yu@uky.edu
Web: http://www.cbme.uky.edu/yu.htm

**NASA**
**Science and Engineering**
**NASA Postdoctoral Fellowships**
NW124416RL
Multiple positions in space and earth science, aeronautics, and astrobiology.
• One-year appointments, renewable up to 3 years • Stipends start at $50,000/yr • Travel budget of $8,000/yr • Financial assistance for relocation and health insurance • Apply at http://nasa.orau.org/postdoc
Application Deadlines: March 1, July 1, and November 1.

**Contact**

Larry Voorhees Tel: 1-865-241-4543
nasapostdoc@orau.org
http://nasa.orau.org/postdoc/

**A POSTDOCTORAL POSITION**
*is available in the lab of*
*Dr. Daniela Cimini at Virginia Tech*

to study mechanisms of chromosome mis-segregation in mammalian tissue culture cells, with particular emphasis on formation, correction, and consequences of merotelic kinetochore attachment. This project is part of a larger collaborative study in which the experimental data will be used to build computational/mathematical models of merotelic kinetochore formation and behavior and mitotic spindle mechanics. Laboratory skills required include tissue culture techniques, microscopy, basic molecular biology and biochemistry. Experience studying cell division or cell motility and performing live-cell imaging is desirable.
Position includes a competitive salary and fringe benefits. Funding is available for one year and possibly more.
**Interested candidates should apply on the Virginia Tech website: http://www.jobs.vt.edu (Posting #071391)**
**Three letters of recommendation should be sent via email to Dr. Daniela Cimini at**
**cimini@vt.edu**
**Review of applications will begin February 1, 2008.**
*Virginia Tech is an Equal Opportunity/Affirmative Action Institution.* NW124515R

dkfz. DEUTSCHES KREBSFORSCHUNGSZENTRUM IN DER HELMHOLTZ-GEMEINSCHAFT

## Postdoc Position

(Ref-No. 13/2008)

**Division: Molecular Genome Analysis**

Tasks/ Description

- A postdoctoral research position is available in the Department of Molecular Genome Analysis at the German Cancer Research Center to study the molecular mechanisms of cancer progression and metastasis.

  Current projects involve integrated studies using 2D and 3D cell culture systems, FACS analysis, RNAi, proteomics and bioinformatics. Successful applicants will have access to state-of-the-art technology including high through-put cell-based assays, cDNA- and protein microarrays, as well as a variety of core facilities.
  The aim of the project is to validate proteins, recently identified as modulators of cell-cell contacts and cell-matrix contacts, in vitro and in vivo.

Profile

- Experience in the following areas is desirable: in vivo animal handling and imaging, measuring the effects of experimental manipulations on the migratory and metastatic behaviour, modern protein and DNA techniques, RNA interference and immunohistochemistry.
- A strong background in genotyping of animals by PCR and Southern-blotting and mouse-anatomy is a must.
- A keen interest in cancer metastasis, to work in an interactive team and good writing and communication skills are expected.

We offer pioneering research projects and optimal working conditions in an international and dynamic scientific environment.

The position is open from April 2008 and limited until 30.09.2009

For further information please contact Dr. Dorit Arlt, phone no. +49 6221/42-4759, e-mail: d.arlt@dkfz.de

DKFZ wish to increase the proportion of female scientists and strongly encourage applications of qualified women. With equal qualifications, handicapped individuals will be considered preferentially.

Applications should be sent to:

Deutsches Krebsforschungszentrum
Personalabteilung
Im Neuenheimer Feld 280
69120 Heidelberg, Germany
or via E-Mail: personalabteilung@dkfz.de
www.dkfz.de

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W123760R

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**Ruprecht-Karls-Universität Heidelberg**

**Medizinische Fakultät Mannheim**

The Centre of Biomedicine and Medical Technology Mannheim (CBTM) is a newly established centre for biomedical research founded by the Medical Faculty Mannheim of the University of Heidelberg that focuses on molecular oncology and vascular biology. The "Microvascular Biology and Pathobiology" research unit headed by Prof. Jonathan Sleeman currently has openings for a number of

## Junior and Senior Postdocs
## PhD students

The laboratory aims to understand the process of metastasis, and in particular the role of the lymphatic system in tumor dissemination. Future research will explore the role of cancer stem cells in metastasis and the importance of pre-metastatic changes in organs in which metastases develop. In addition to generous University financing, these activities will be supported by EU FP7 funding under the auspices of the TuMIC Collaborative Project that is coordinated by the laboratory. An additional position is funded by the AICR. More information can be obtained at **www.ma.uni-heidelberg.de**

Commensurate with their stage of academic development, successful candidates will be expected to have an excellent grounding in molecular and cellular biology, together with a good understanding of molecular oncology and/or vascular pathobiology. Experience in tumor angiogenesis, lymphangiogenesis and metastasis research, the use of animal models (including genetically modified mice), FACS sorting, single cell cDNA library techniques and/or methods for the manipulation of endothelial cells in vivo and ex-vivo would be an advantage.

Potential applicants are warmly invited to make informal enquiries (sleeman@medma.uni-heidelberg.de). Candidates should supply a detailed CV (including a list of any publications), a short summary of their research experience and interests, and the names and contact details of two or three referees. Applications should be sent either electronically as a single PDF file to sleeman@medma.uni-heidelberg.de, or by post to **Prof. Jonathan Sleeman, Universität Heidelberg, Medizinische Fakultät Mannheim, Tridomus-Gebäude Haus C, Ludolf-Krehl-Str. 13-17, D-68167 Mannheim, Germany.** There is no formal closing date for applications, but candidates are strongly encouraged to submit their applications within six weeks of the publication of this advertisement.

The University of Heidelberg seeks to increase the proportion of female scientists and strongly encourages applications from qualified women. In the case of equivalent qualifications, handicapped individuals will be considered preferentially.

W123652R

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**Medical College of Wisconsin**

**POSTDOCTORAL STUDY**

Investigators in a growing immunology group at the Medical College of Wisconsin are recruiting for two or more Post-doctoral positions to study: (i) molecular basis for the immunogenicity of Adenovirus E1A (J Exp Med. 202(11): 477-82, 2005); (ii) role of DNA tumor viruses in lymphoproliferative disorders in patients with Primary Immune Deficiencies (J Exp Med.15;202(4): 479-84, 2005) and (iii) the molecular basis for B cell anergy and the role of B cells in diseases of immune dysregulation (Immunity. 2006 Dec;25(6):864-7. Nat Immunol. 2005 Nov;6(11):1072-4). A Ph.D. with a strong background in molecular biology and immunology is required.

To apply, visit www.mcw.edu/careers and apply for position #350.17790 or #350.18313

MEDICAL COLLEGE OF WISCONSIN

EEO/AA M/F/D/V

NW124426R

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MRC

**MRC Functional Genetics Unit**

**Comparison of Metazoan Genomes**

**Career Development Fellowship**

A three year BBSRC-funded position is available immediately in Prof Chris Ponting's group. This post will apply computational genomic approaches to predict protein-coding and non-coding functional sequence, from diverse metazoans, using the neutral indel model (PLoS Comp Biol 2:e5). Candidates should have a strong background in bioinformatics.

**Contact**

MRC Recruitment (Ref FGU08/068)
Tel: 01793 301156
Web: www.mrcfgu.ox.ac.uk

U124345RL

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MANCHESTER 1824

The University of Manchester

**Faculty of Life Sciences**

## Postdoctoral Research Associate in the Molecular and Cellular Biology of Biopharmaceuticals Production

**Ref: LS/017/08**

**£26,666 - £32,796 p.a.**

This position is based within a group that forms part of the BBSRC Industry sponsored Bioprocessing Research Industry Club (BRIC) initiative. The project title is "Application of metabolomics profiling of recombinant mammalian cells to bioprocess design".

Further details can be found at: www.bbsrc.ac.uk/science/initiatives/bric

You will be able to work effectively as part of a team, within this University and in interactions with colleagues in the wider BRIC network of academic and industrial collaborators.

You will hold a degree in science and a PhD in a bioscience subject. You will have primary responsibility for analytical technologies and predictive modelling approaches but flexibility of approach, and a desire to contribute to all aspects of the programme (covering molecular biology, cell physiology and chemical engineering).

The position is tenable from 1 April 2008 until 31 March 2010.

Informal enquiries may be made to: Professor Alan Dickson on +44 (0) 161 275 5077 or alan.dickson@manchester.ac.uk

**Application forms and further particulars can be obtained at our website or by contacting +44 (0) 161 275 8836 or lifesciences-hr@manchester.ac.uk quoting the reference number.**

**Closing date: 21 February 2008.**

**Cardiovascular Research Group**

## Research Associate

**Ref: MHS/039/08**

**£26,666 - £28,289 p.a.**

You will study the formation of an intra-luminal sarcoplasmic recticulum (SR) calcium sensor. Mis-regulation of calcium release from the SR is linked to the pathogenesis of heart failure and fatal arrhythmias.

This project will employ a structural biology approach (in conjunction with molecular biology and biochemical methods) to probe the dynamics and assembly of the various protein components proposed to form the sensor to correlate to functional observations. This project is closely allied with other on-going projects.

The work will involve purification of the SR ryanodine receptor (RyR) from native tissue, overexpression and purification of protein domains in E.coli for examination using cryo-electron microscopy and single particle analysis methods.

For informal enquiries please contact Dr. A. Kitmitto on +44 (0) 161 306 4186 or ashraf.kitmitto@manchester.ac.uk

This position is tenable from 1st March 2008 for up to 2 years.

**Application forms and further particulars are available from our website or by contacting +44 (0) 161 275 1197 or julie.a.heydon@manchester.ac.uk quoting the reference number.**

**Closing date: 22 February 2008.**

**School of Cancer & Imaging Sciences**

## Research Associate

**Ref: MHS/040/08**

**£26,666 – £31,840 p.a.**

You will work on an externally funded project studying specific molecular features of childhood brain tumours. It is a multidisciplinary project applying molecular biology expertise to studies on the altered transcription resulting from the disruption of the DNA damage response in childhood brain tumours. You will require a PhD and experience in gene expression analysis. Experience in the field of DNA damage response is desirable.

The post is available for 18 months in the first instance and is tenable immediately.

Informal enquiries can be made to Dr Stefan Meyer on + 44 (0) 161 446 3094 or stefan.meyer@manchester.ac.uk

**Application forms and further particulars are available from our website or by contacting +44 (0) 161 275 8835 or mhs-hr@manchester.ac.uk quoting the reference number.**

**Closing date: 22 February 2008.**

*The University will actively foster a culture of inclusion and diversity and will seek to achieve true equality of opportunity for all members of its community.*

U124598R

www.manchester.ac.uk/jobs

# University of Oxford

## Postdoctoral Researchers - Oxford Centre for Integrative Systems Biology

The OCISB is working to develop interdisciplinary approaches to model, initially, microbial networks and interactions. The groups involved span the major Science Departments of the University of Oxford. Our aim is to understand, predict and control physiological behaviour by integrating knowledge of interactions at molecular, cellular and population levels.

Dramatic advances in biotechnology have led to the mapping of the complete genomes of many organisms. This information, however, is of limited value because biological function arises out of the interaction of components. So, not only must we identify the component parts, we must also investigate how they link to one another. This is the 'Grand Challenge' of Systems Biology and is a major scientific challenge in the post-genomic era. To understand how these systems interact we need to use computational and mathematical approaches. We feel that if we cannot do this for the simplest, most well-characterised system, then we will be unable to do it for more complex systems.

We have up to 10 postdoctoral positions available and are looking for researchers in areas including:

- Meiotic regulatory networks, hypoxia responses, bacterial chemosensory networks
- Image analysis at different scales
- Computational and Mathematical modelling to produce integrative models.

There are also positions available in Data Management.

**For further details please see our website www.sysbio.ox.ac.uk/opportunities**

**The University are Equal Opportunity Employers.**
**We positively encourage**
**applications from people of all backgrounds**

U124571RM

www.ox.ac.uk/jobs

Birkbeck is a world-class research institution, a vibrant centre of academic engagement and excellence and the UK's leading provider of part-time, evening education for mature students.

Birkbeck
UNIVERSITY OF LONDON

**Educating Busy Londoners**

## Postdoctoral Research Assistant – *Ref: ACR211*

### School of Crystallography, Faculty of Science

### Full-time, fixed term appointment for up to 12 months

We are seeking a talented individual to provide research assistance on a Heptagon-funded project to work with Professor Bonnie Ann Wallace in the School of Crystallography.

The project "Prokaryote/Eukaryote Chimeras for Membrane Protein Expression in E Coli" will focus on cloning, expression, purification, and structural and functional characterisation of ion channels.

Applicants for this post should possess good skills in molecular biology and biochemistry (preferably of membrane proteins), and will have completed a PhD and (preferably) have postdoctoral experience in a closely related area.

**Salary range will be from £30,977 to £43,023 per annum inclusive of London Allowance on Grade 7 or 8, initial salary will be dependent on the skills and experience of the successful applicant.**

For an application form and further details on each post, please visit the Birkbeck website at http://www.bbk.ac.uk/hr or send an A4 sae (quoting reference ACR211) to the Human Resources Team, Birkbeck, Malet Street, Bloomsbury, London WC1E 7HX or email humanresources@bbk.ac.uk

**Closing date: 25 February 2008**

Birkbeck is an equal opportunities employer.

U124576R

"Advertising an open high profile position Nature jobs resulted in outstanding group of highly qualified applicants from all over the world within a very short period of time.
This efficient service is our first choice for scientific recruitment."

– Dr Obrecht Jean-Pierre,
Polyphor AG, Switzerland

# Chief Scientist to head a new physics laboratory
# Permanent Position, RIKEN

RIKEN invites applications for the position of Chief Scientist to head a new laboratory. Applications from overseas applicants are welcome. The successful candidate will be responsible for the laboratory's overall management and research strategy, directing research projects and contributing to more general aspects of RIKEN's management and research planning activities.

**Laboratory**

The laboratory program will explore the atomic physics in wide sense, namely the broad area of physics over a wide scale (i.e., interdisciplinary studies in atomic, nuclear, particle and astro-physics) underlying the genesis/evolution of the universe. The laboratory is expected to be synergistic and complementary to other laboratories in RIKEN.

**Job title and number of positions**

Chief Scientist, one person.

**Qualifications**

Applicants should have ability and experience equivalent to that of a professor who manages research at a university graduate school, and appropriate research experience supported by a distinguished research record and the ability to play a pivotal role in these areas. This position is open to all nationalities.

**Status**

The post is a permanent appointment, subject to RIKEN's mandatory retirement age of 60. However, it is possible, depending on evaluation results, to continue research after the age of 60 (73 maximum) as a Distinguished Senior Scientist. Terms and conditions of employment shall include a director-level salary and be in accordance with RIKEN's procedures for appointing Chief Scientists.

**Deadline and documents to be submitted**

Applicants should send a full curriculum vitae and photograph; list of publications; one copy each of five key publications; a statement (about five pages A4 sized paper) explaining former research experience, and proposals for research at RIKEN; and the names and addresses of three referees. All applications should reach RIKEN by 31 March 2008.

**Personal information**

Submitted documents will be handled in accordance with the RIKEN rules concerning personal information, and only used for screening applications for this position. Personal information will not be disclosed, transferred, or lent to any third party without a justifiable reason.

JP12420GR

**Starting date**

October 1, 2008 or as soon as possible after that.

**Note**

Submitted documents will normally not be returned. Information about RIKEN and its procedures for appointing Chief Scientists is available on the RIKEN website: http://www.riken.jp/

**Inquiries, and address to which applications should be submitted**

Dr. Masahiko Iwasaki, Head of the Chief Scientist Nominating Committee, Advanced Meson Science Laboratory, RIKEN, 2-1 Hirosawa Wako-shi, Saitama, 351-0198, JAPAN
Tel: +81-48-467-9352 / Fax: +81-48-462-4648
E-mail address: advanced_meson@riken.jp

## UNIVERSITY OF KONSTANZ

**Konstanz Research School – Chemical Biology (KoRS-CB): Research and Graduate Training at the Interface of Chemistry and Biology**

The newly founded »Konstanz Research School - Chemical Biology« (KoRS-CB) is an interdisciplinary initiative of the Departments of Biology, Chemistry, and Computer & Information Science at the University of Konstanz and is supported by the German Excellence Initiative.

The main objective of KoRS-CB is to guide talented graduate students to scientific excellence in an area that is highly relevant for both basic and applied research. The research program of KoRS-CB comprises the research areas Synthetic Chemistry, Cellular Biochemistry, Biophysics, Biomedicine and Computational Biology.

KoRS-CB will commence its training program in **April 2008**. Thus, KoRS-CB invites applications for

### Fellowships for Ph.D. students

from highly motivated and enthusiastic students with a keen interest in interdisciplinary research and an excellent degree (Master or Diploma) in Biology, Chemistry or related areas.

The University of Konstanz is a true campus university located on spacious grounds within one of the most beautiful areas of Germany overlooking Lake Constance and close to the Alps. The University of Konstanz is one of nine German universities that have been awarded the status of a »University of Excellence« by the German Excellence Initiative and provides state-of-the-art research facilities for interdisciplinary and cutting-edge research.

For details on the application procedure and further information on the research and training program of KoRS-CB, the participating Departments and the University of Konstanz, please visit the KoRS-CB homepage at
http://www.chembiol.uni-konstanz.de

For further information please visit our homepage:
**http://www.uni-konstanz.de/stellen**

W124352R

Medizinische Fakultät Heidelberg

An der Universitäts-Hautklinik der Medizinischen Fakultät der Ruprecht-Karls-Universität Heidelberg ist zum nächstmöglichen Zeitpunkt eine

### W3-Professur für Immundermatologie

zu besetzen. Der/Die Stelleninhaber/in wird das Fach Dermatologie und Venerologie in seiner ganzen Breite mit Schwerpunkt „Immunologische Krankheiten der Haut" vertreten. Es ist geplant, dem/der Stelleninhaber/in die Position des/der Leitenden Oberarztes/-ärztin zu übertragen.

Einstellungsvoraussetzungen sind die Anerkennung zum Facharzt für Dermatologie und Venerologie sowie die Habilitation für das Fach Dermatologie oder eine gleichwertige Qualifikation. Es werden Leitungserfahrungen in der klinischen Dermatologie, der Betreuung von Patienten mit Hauttumoren und herausragende wissenschaftliche Leistungen auf dem Gebiet der Immuntherapie entzündlicher Hauterkrankungen und der Allergologie erwartet. Ferner soll der Bewerber in der dermatologisch-immunologischen Grundlagenforschung ausgewiesen sein.

Der/Die Bewerber/in soll aktiv am Forschungsschwerpunkt Immunologie und Transplantation der Medizinischen Fakultät der Universität Heidelberg partizipieren. Hierunter wird auch die Mitarbeit an neu zu gründenden Sonderforschungsbereichen oder Forschergruppen sowie der Exzellenzinitiative verstanden. Ein Schwerpunkt der Forschung der Hautklinik ist hierbei die Immunregulation und die Biologie dendritischer Zellen. Ferner gehören eine aktive Drittmitteleinwerbung und Engagement in der akademischen Selbstverwaltung der Fakultät zum Aufgabenbereich des Bewerbers. Angesichts des neuen klinischen Curriculums HEICUMED wird von dem Bewerber ein überdurchschnittliches Engagement in der Lehre erwartet.

Die Stelle steht unbefristet zur Verfügung. Bei der ersten Berufung in ein Professorenamt ist jedoch das Dienstverhältnis gem. § 50 Abs. 1 Landeshochschulgesetz grundsätzlich zu befristen. Ausnahmen sind insbesondere möglich, wenn Bewerber aus dem Ausland oder aus einem Bereich außerhalb der Hochschulen sonst nicht gewonnen werden können. Soll das Dienstverhältnis nach Fristablauf fortgesetzt werden, bedarf es nicht der erneuten Durchführung eines Berufungsverfahrens.

Die Universität Heidelberg strebt eine Erhöhung des Anteils der Frauen beim wissenschaftlichen Personal an und fordert qualifizierte Frauen nachdrücklich auf, sich zu bewerben. Schwerbehinderte werden bei gleicher Qualifikation bevorzugt eingestellt.

Bitte richten Sie Ihre Bewerbung innerhalb von **4 Wochen** nach Erscheinen der Anzeige an **Prof. Dr. C. R. Bartram, Dekan der Medizinischen Fakultät Heidelberg, Im Neuenheimer Feld 672, 69120 Heidelberg.** Ihre Bewerbungsunterlagen sollten den Kriterien entsprechen, welche Sie unter Dekanat@med.uni-heidelberg.de anfordern können.

W123945R

# National Climate Change Adaptation Research Facility Director

Griffith UNIVERSITY

Australian Government
Department of Climate Change

- Strategic multidisciplinary research leadership
- New national facility, exciting Gold Coast location

The National Climate Change Adaptation Research Facility is part of the $126 million Australian Government investment in climate adaptation managed by the Australian Government Department of Climate Change. The Facility is charged with leading the Australian research community to generate the biophysical, social and economic information needed to manage the effects of climate change.

The Facility will be hosted by Griffith University in partnership with the Queensland Government and eight universities across Australia. A research intensive institution with a reputation for innovation and responsiveness to change, Griffith researchers established a Climate Change Response Program in 2006 to address fundamental issues of adaptation and response to climate change. This Program will provide strong support for the Director of this Facility.

The Director will drive the development and implementation of National Adaptation Research Plans. The successful candidate will work closely with the Department of Climate Change to integrate research leadership with policy development, program delivery and stakeholder engagement, and to build strategic and collaborative relationships with government, industry, business and community.

A distinguished research record in a field relevant to NCCARF's research agenda, and demonstrated high-calibre leadership capabilities relevant to climate adaptation are essential requirements. Extensive experience in managing complex research processes and in building research collaborations between researchers, partners and stakeholders is expected. Outstanding interpersonal and communication skills, with the stature and expertise to build key relationships across government, industry and adaptation research communities will be supported by a commitment to excellence and outcomes.

**Initial enquires can be made in confidence to Christine Ryder on +61 2 9335 8640. Applicants are requested to write a claim for the position and this will be assessed against the selection criteria. To apply, email this claim, your resume and names of three referees to execrecruitment@kpmg.com.au quoting reference number 71563, fax to +61 2 9335 7020. For further information: http://www.griffith.edu.au/hrm/employment/ Closing date for applications is Friday 22nd February 2008.**

JP124332R

KPMG

SINAPSE
Scottish Imaging Network - A Platform for Scientific Excellence

# 5 Chairs in Imaging

SINAPSE is an alliance of six universities which, in partnership with the Scottish Funding Council and Chief Scientist's Office of the Scottish Government Health Department, is creating a shared, comprehensive, network for human neuroimaging research incorporating Magnetic Resonance, Molecular and Electrophysiology imaging techniques to meet the emerging opportunities and challenges primarily posed by clinical neuroscience research in health and disease.

**3 Research Professors in Neuroimaging (non clinical)**

SINAPSE seeks to appoint 3 outstanding Research Professors in Imaging physics, Neuroimaging, Functional Imaging, Molecular Imaging or Image Analysis to augment the established strengths in these themes.

**2 Clinical Research Professors in Imaging**

We also seek 2 outstanding Research Professors in neuro, vascular or oncology imaging to establish imaging research programmes that complement the considerable existing clinical research strengths in these disciplines.

Successful applicants will have established internationally excellent research in imaging and will be expected to make significant contributions to enable international quality research in Scotland. These positions may be held at any appropriate University within the SINAPSE consortium.

Potential applicants must contact those Universities whose research complements their own to discuss their interests and arrange a visit.

University of Aberdeen: Professor Mike Greaves,
e-mail: m.greaves@abdn.ac.uk, or tel: +44 (0)1224 553015.

University of Dundee: Professor Mike Coughtrie,
e-mail: m.w.h.coughtrie@dundee.ac.uk or tel: +44 (0)1382 632166.

University of Edinburgh: Professor Joanna Wardlaw,
e-mail: jwardlaw@staffmail.ed.ac.uk or tel: +44 (0)131 5372943.

University of Glasgow: Professor John Coggins,
e-mail: j.coggins@admin.gla.ac.uk or tel: +44 (0)141 3308137.

University of St Andrews: Professor Alan Miller,
e-mail: vpres@st-andrews.ac.uk or tel: +44 (0)1334 462525.

University of Stirling: Professor Ian Simpson,
e-mail: i.a.simpson@stir.ac.uk or tel: +44 (0)1786 467013.

**For further particulars, including details of universities' interests, and general application enquiries please visit www.sinapse.ac.uk**

**Applications are being received on behalf of SINAPSE by FWB Executive Search Specialists. To request an application pack or submit a formal application, email: enquiries@fwbltd.com or contact Rhona Armstrong on 0131 539 7087**

**Closing date for full applications: 30 April 2008.**

Committed to Equality and Diversity

CHIEF SCIENTIST OFFICE

UNIVERSITY OF ABERDEEN · UNIVERSITY OF DUNDEE · THE UNIVERSITY OF EDINBURGH · University of St Andrews · University of Glasgow · UNIVERSITY OF STIRLING · Scottish Funding Council

U124597R

## UNIVERSITY OF COLOGNE

The Medical Faculty of the University of Cologne invites applications for the following position:

## Independent Junior Research Group "Molecular Infectiology" Group Leader Position
**(salary scale TV-L-15)**

The candidate, a highly qualified junior scientist will be an expert in Innate Immunity with preferred research interest in mammalian host cell responses to intracellular pathogens. We expect relevant postdoctoral research experience, an excellent scientific record, the ability to independently lead a research group, active collaboration and significant contributions to the research goals of the Collaborative Research Center (SFB) 670 "Cell-autonomous immunity".

The Independent Junior Research Group will co-operate with the general research programme of the SFB 670 (for further information see www.sfb670.uni-koeln.de) and be located at the Institute for Medical Microbiology, Immunology and Hygiene, where the required laboratory/office space and basic equipment will be provided.

The Independent Junior Research Group is funded for five years and will enable independent research to be carried out within a research network. Funding includes the position of the group leader, 1 postdoc, 1 PhD student and 1 technican as well as consumables and instrumentation. For further information please contact martin.kroenke@uk-koeln.de.

Female scientists are particularly encouraged to apply and will be preferentially considered if suitable qualified.

We also welcome applications from disabled candidates, who also will be preferentially considered if suitable qualified.

Applications should include a research plan (max. 3 pages), CV, list of publications and copies of the most important publications to be submitted by **March 13, 2008** to the **Dean of the Medical Faculty of the University of Cologne, 50924 Köln, Germany.**

W124396R

## The University of Edinburgh

**The University of Edinburgh is an exciting, vibrant, research-led academic community offering opportunities to work with leading international academics whose visions are shaping tomorrow's world.**

## Senior Academic Fellow

**£33,779 – £40,335 or £42,791 – £48,161**

Working in the School of Biomedical Sciences in the area of Pharmacology, this post will be for a period of five years, starting in August 2008 and leading to a subsequent academic appointment, subject to satisfactory progress. It is an ideal position for a motivated researcher, and will allow a focus on research with limited teaching duties in the early years of the appointment.

You should have a PhD or equivalent experience. Preference will be given to those who have a proven record in applying for, and success in obtaining, research funding.

Informal enquiries to Professor A J Harmar (head.sbms@ed.ac.uk).

**Apply online, view further particulars or browse more jobs at our website. Alternatively, telephone the recruitment line on 0131 650 2511. Ref: 3008571NA. Closing date: 28 February 2008.**

Committed to Equality and Diversity

www.jobs.ed.ac.uk

U124570R

# Babraham Institute PhD Student Opportunities in Ageing Research 2008

Babraham Institute

The Babraham Institute is an international focus for innovative research in post-genomics studying gene function in cells, organs and systems, supported principally by the Research Councils. It is a recognised postgraduate teaching Department of the University of Cambridge. To support our new Institute Initiative in Ageing Research three BBSRC Targeted Priority Studentships will be available at Babraham, starting from October 2008, leading to a University of Cambridge PhD degree in the area of "Molecular and cellular mechanisms regulating ageing". Babraham Science is uniquely placed to make significant inroads into the fundamental molecular and cellular biology mechanisms that are responsible for the ageing phenotype. Babraham is a recognised centre of excellence in areas of signalling, genomic and epigenetic research, employing integrative in vivo (e.g. genetically/nutritionally modified), ex vivo (e.g. adult stem cell) and in vitro models. The three studentships can be awarded for up to four years and are in addition to our normal Quota Studentships allocated via our Open Day.

Eligibility for Funding: Please consult the following website (http://www.bbsrc.ac.uk/funding/training/eligibility.pdf) for details of eligibility and funding. Students will join a thriving scientific community situated on an attractive parkland campus near Cambridge. Our 70 students are all members of Cambridge Colleges and participate fully in University social and academic life (www.bio.cam.ac.uk/gradschool).

Details of our scientific programmes can be found on www.babraham.ac.uk. The Institute is fully equipped for state-of-the-art biological research including: innovative molecular biology, stem cell manipulation and transgenics, real-time laser scanning confocal microscopy, fluorescence sorting of cells, gene targeting and knockouts, mouse models of disease, mouse behavioural testing and proteomics. Selected students will be invited to attend interviews, discuss their research interests and view the Institute's facilities.

Potential projects are listed below and further details of each project are also given on the Student pages of our website (www.babraham.ac.uk); supervisors welcome informal enquiries:

Epigenetics of foetal programming
**Dr Gavin Kelsey**, gavin.kelsey@bbsrc.ac.uk

Foetal programming of cardiac health
**Dr Llewelyn Roderick**, llewelyn.roderick@bbsrc.ac.uk

Regulation of cardiac myocyte remodelling
**Dr Martin Bootman**, martin.bootman@bbsrc.ac.uk

Axonal transport of mitochondria during ageing
**Dr Michael Coleman**, michael.coleman@bbsrc.ac.uk

PI3Kinase and ageing-related changes in the immune system
**Dr Klaus Okkenhaug**, klaus.okkenhaug@bbsrc.ac.uk

The role of Akt in adult muscle stem cells
**Dr Jenny Pell**, jenny.pell@bbsrc.ac.uk

Autophagy and age-dependent regenerative potential of stem cells
**Dr Nicholas Ktistakis**, nicholas.ktistakis@bbsrc.ac.uk

Travel expenses will be paid to those invited for interview. Applicants should submit a full Curriculum Vitae with a covering letter indicating the two projects in which they are most interested, in order of preference, and ask two referees to write to the Institute on their behalf before the deadline.

Please send your applications to: Ms Linda Notton, Graduate Studies Programme, The Babraham Institute, Babraham, Cambridge CB22 3AT, Tel: 01223 496338, Fax: 01223 496022 or email babraham.graduate@bbsrc.ac.uk by **FRIDAY 15th FEBRUARY 2008**.

An Institute supported by the Biotechnology and Biological Sciences Research Council. An Equal opportunities employer.

**www.babraham.ac.uk**

U124083R

nature

# Managing Editor

*Nature* is the world's leading weekly scientific journal and is the flagship publication of Nature Publishing Group.

With its authoritative journalism and opinion, a leading position in its science research content, and worldwide influence and engagement, *Nature* stands ready to undertake a period of further investment in both print and online formats. The publisher and the Editor-in-Chief of *Nature* wish to employ a senior manager who will take direct responsibility for the implementation of the publishing programme and for key aspects of publishing and editorial management.

Applicants must have a demonstrable familiarity with the scientific landscape, strong commercial drive, and the ability to manage projects and to achieve demanding goals in a way that stimulates and inspires the colleagues on whom they depend.

The job is based in the London offices of the Nature Publishing Group (NPG), and involves close interactions with colleagues in other parts of Europe, the United States and the Asia-Pacific. The Managing Editor will report to the Editor-in-Chief of *Nature* and to the Managing Director of NPG.

Candidates should have a strong commercial drive, prior editorial experience, preferably in scientific publishing, and some previous experience of the commercial side. They should be comfortable with print and online media and have experience of running projects and managing teams.

The position will be full-time.

The position is based in our modern London offices.

**Please send your CV, a summary of relevant experience, and your current salary, quoting reference number to NPG/LON/815, to Geetika Juneja Personnel Assistant at londonpersonnel@macmillan.co.uk**

All candidates must demonstrate the right to live and work in the UK to be considered for the vacancy.

*Closing Date: 14th February 2008*

nature publishing group npg

IN123677R

nature materials

# Associate Editor

*Nature Materials* is a prestigious international monthly journal (Impact Factor 19.194) covering all aspects of materials science and technology. We have an exciting opportunity available for a materials scientist or a chemist to join our editorial team as an Associate Editor working on all aspects of the journal.

We are particularly interested in applicants with expertise in physical chemistry and soft matter research but we would welcome applications from outstanding candidates in any area of materials science.

The ideal candidate should have a PhD and preferably postdoctoral experience with a strong research record. The successful candidate will play an important role in determining the representation of their field in the journal, and will work closely with the other editors on all aspects of the editorial process, including manuscript selection, commissioning and editing of Reviews and News & Views, and writing for the journal. A key aspect of the job is liaising with the scientific community through laboratory visits and international conferences.

This is a demanding and intellectually stimulating position. Broad scientific knowledge and training, excellent literary skills and a keen interest in the practice and communication of science are a prerequisite. The successful candidate must, therefore, be dynamic and outgoing and have excellent interpersonal skills. The salary and benefits will be competitive, reflecting the critical importance and responsibilities of this position.

The new editor will join our team in our London office. The *Nature Materials* team is part of a dynamic editorial and publishing environment that also includes *Nature*, *Nature Physics* and *Nature Nanotechnology*.

Applicants should send a CV (including their class of degree and a brief account of their research and other relevant experience), a News & View style piece (600 words or less) on a recent paper from related literature, and a brief cover letter explaining their interest in the post and their salary expectations.

*The closing date for applications is Monday 25th February 2007.*

**To apply please send your CV and covering letter, quoting reference number NPG/LON/823 to Denise Pitter at londonrecruitment@macmillan.co.uk**

All candidates must demonstrate the right to live and work in the UK to be considered for the vacancy.

nature publishing group npg

IN124059R

ikerbasque

Basque Foundation for Science

# *International Call for Researchers*

*Ikerbasque, the Basque Foundation for Science, promoted by the Basque Government offers* **30 positions** *for senior researchers.*

- **Ikerbasque has launched an international call for researchers in 2008, in order to contract 30 senior researchers who have a:**
  - Ph. D. before January 2004
  - Solid research track
- **Terms and conditions**
  The researchers will carry out their investigation in centres in the Basque Science System, preferably within a university
- **Deadline**
  There will be 2 evaluations, one for all the applications received before 31st March, and another for the applications received between 1st April and 30th of September
- **Aplications at**

**www.science.eu.com**

EUSKO JAURLARITZA
GOBIERNO VASCO

BASQUE GOVERNMENT
Department of Education, Universities and Research

W124208R

www.cardiff.ac.uk/jobs

CARDIFF UNIVERSITY
PRIFYSGOL CAERDYDD

# Non Clinical Lecturer in Neuroimaging

## Department of Psychological Medicine
## School of Medicine

The department is launching a new initiative in neuroimaging and affective neurosciences, headed up by Professor Mary Phillips, which will complement existing strengths in psychiatric genetics, animal models and epidemiology. This will benefit from the recent investment of £10m in neuroimaging to develop CUBRIC (http://www.cardiff.ac.uk/psych/cubric/index.html), a dedicated research facility equipped with state-of-the-art 3T-MRI, MEG, EEG, ERP and TMS for human studies. You will be expected to have a PhD and previous experience in research at a post-doctoral level and will be encouraged to prepare and submit applications for external funding. You will also be expected to play a role in the department's teaching programmes including the undergraduate curriculum and the MSc in Psychiatry.

This post is fixed-term for 5 years.

**Salary: £34813 - £40335 per annum**

**Informal enquiries can be made to Professor Mary Phillips, email phillipsM10@cardiff.ac.uk to discuss the appointment further.**

**To work for an employer that values and promotes equality of opportunity, visit www.cardiff.ac.uk/jobs telephone + 44 (0) 29 2087 4017 or email vacancies@cardiff.ac.uk for an application form quoting vacancy number 069.**

**Closing date: 28 February 2008.**

U124553R

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NANYANG TECHNOLOGICAL UNIVERSITY

NUS National University of Singapore

# LEE KUAN YEW POSTDOCTORAL FELLOWSHIP

Applications are invited from young and outstanding academics for the prestigious Lee Kuan Yew Postdoctoral Fellowship (LKY PDF) in the National University of Singapore (NUS) and Nanyang Technological University (NTU).

The Fellowship is tenable for up to three years, with possible extension for two further years. LKY PDFs can apply for academic positions following the Fellowship.

Gross annual salary ranges from S$72,000 to S$144,000 (approx US$50,000 to US$100,125) with commencing salary depending on qualifications and experience. Leave and medical benefits will be provided.

For details of other benefits offered and application procedure, please visit the websites of the respective University*.

## APPLICATION

Interested candidates should send their complete application package, comprising all documents listed below, to the respective University*.

- NTU Application or NUS Personal Particulars Form (downloadable from website)
- Detailed Curriculum Vitae, List of Publications and Educational Certificates
- Three International Referee Reports (including contact details)
- Statement of Research Intent (details of proposed research plan)

**Closing date: 5th March 2008**

**Successful candidates will be notified in June 2008**

*For application and contact details, please see
**NUS: http://www.nus.edu.sg/ore/fellowships/fellowship_lky.htm**
**NTU: http://www.ntu.edu.sg/hr/recruit/research/LKY2008.htm**

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University of Glasgow

## Dean of Physical Sciences

We are seeking an outstanding individual – a leading academic who is passionate about science with a strong record of academic achievement, and a confident and enthusiastic leader who will enjoy the opportunity to direct a large faculty in a Russell Group university.

Physical Sciences at Glasgow comprises Chemistry, Geographical & Earth Sciences, and Physics & Astronomy. As Dean, you would lead the Faculty's strategic direction and manage its financial, human and physical resources. You would enhance its reputation in research and teaching, maintain its healthy resource base and build strategic partnerships. As a member of the Senior Management Group, you would help set the University's strategy for future growth and be part of the corporate team leading one of the world's top 100 universities.

Informal enquiries may be made to Professor Steve Beaumont, Vice-Principal on +44 (0)141 330 2112 or email s.beaumont@enterprise.gla.ac.uk

For further information on Physical Sciences at Glasgow, go to www.glasgow.ac.uk/faculties/physicalscience

The closing date for applications is 29 February 2008.

Interviews will be held on 7/8 April 2008.

For an application pack, contact Tracey Stirling, Human Resources Department, University of Glasgow, Glasgow G12 8QQ, Tel: 0141 330 2913, E-mail: t.stirling@admin.gla.ac.uk

The University is committed to equality of opportunity in employment.

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# Four-year MRC PhD Studentships

## MRC Centre for Transplantation at King's College London

King's College London wishes to appoint six studentships to join the MRC Centre for Transplantation. We are seeking to appoint outstanding science graduates who will engage in internationally-competitive research projects led by recognised experts. The first year will incorporate a rotation through several of these areas focusing on generic skills, followed by three years in a chosen topic. The projects available are:

**Project 1:** In vivo imaging of T-Regulatory cell mediated transplant tolerance.
**Project 2:** Immunosurveillance by the transitional immune system.
**Project 3:** Statistical analysis of genome-genome interaction with reference to kidney transplant outcome.
**Project 4:** Immune response to dental cell implants.
**Project 5:** Studies of skin transplantation in humanized mice.
**Project 6:** Complement induced T-regulatory lymphocytes: triggers and signalling pathways

Candidates must possess, or be expected to achieve, a first or good upper second class degree in a relevant subject and must meet MRC residency requirements.

For further details on projects and how to apply please visit **www.kcl.ac.uk/schools/medicine/mrcstudentships.html** or contact **mrccentre@kcl.ac.uk**

U124397R

MRC Centre for Transplantation

KING'S College LONDON

*Equality of opportunity is College policy*

# Imperial College London

Department of Mathematics

## Lectureship in Biomathematics

From 1 May 2008, the salary for this post will be in the range of £40,050 to £44,730 per annum

Imperial College is ranked the fifth best university in the world (Times Higher QS World University Rankings 2007).

Applications are invited for a Lectureship in Biomathematics within the Department of Mathematics at Imperial College London.

Collaboration between biomedicine and the mathematical sciences is a very high priority throughout the whole of Imperial College London. Within the Department of Mathematics, this has been reflected by the recent appointments of a chair and a lectureship in this field and the establishment of the Biomathematical Sciences research group.

You will have a PhD in the Mathematical Sciences or closely related discipline. You will be expected to demonstrate an outstanding record of research in any area of biomathematics, systems biology, biophysics or similar field. You will also have experience of collaboration with experimental biologists.

The post is available from 1 October 2008 or as soon as possible thereafter.

An application form and further particulars can be obtained from http://www.imperial.ac.uk/employment/academic/index.htm Alternatively, please contact Mr Kalra Taylor, email: k.taylor@imperial.ac.uk tel: +44 (0)20 7594 8483.

To apply, please send a copy of your application form together with your CV, a list of publications and names of three referees by email to Mr Kalra Taylor (k.taylor@imperial.ac.uk).

**Closing date: 7 March 2008.**

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U124573RM

# The University of Edinburgh

**The University of Edinburgh is an exciting, vibrant, research-led academic community offering opportunities to work with leading international academics whose visions are shaping tomorrow's world.**

## Senior Research Fellow/Reader

**£42,791 – £48,161**

The renowned Roslin Institute is joining forces with the University of Edinburgh to create a new world-leading research organisation in the animal sciences. The new Institute will be operational from April 2008 and incorporate leading scientists from the Roslin Institute, The University of Edinburgh and the Scottish Agricultural College.

We require scientists whose experience will complement the existing strengths and the science strategy of the Institute. Appointments will be made at Senior Research Fellow or Reader level and scientists with an interest in the following areas are encouraged to apply:

- genetics and genomics
- stem cells, differentiation and development
- tissue growth, homeostasis and ageing
- animal models of disease
- immunity to infectious disease
- inflammation
- neuroscience and neuropathogenesis
- molecular neuroendocrinology

Although the focus and strength of the Institute lies with companion and livestock animals as systems, there is no requirement that you currently focus specifically on problems that affect animals. Key requirements are a strong track record of publication and grant funding (appropriate to level of experience), evidence of leadership and a focus that complements existing strengths and the science strategy of the Institute.

Informal enquiries to David Rigby, Human Resources, or in particular research areas Bruce Whitelaw, Development (e-mail: bruce.whitelaw@roslin.ed.ac.uk), Alan Archibald, Genetics and Genomics (e-mail: alan.archibald@roslin.ed.ac.uk), Ivan Morrison, Immunity (e-mail: ivan.morrison@ed.ac.uk) or Jean Manson, Neuroscience and Neuropathogenesis (e-mail: jean.manson@roslin.ed.ac.uk).

**Apply online, view further particulars or browse more jobs at our website. Alternatively, telephone the recruitment line on 0131 650 2511. Ref: 3008583NA. Closing date: 7 March 2008.**

Committed to Equality and Diversity

**www.jobs.ed.ac.uk**

U124590R

# Massey University Postdoctoral Fellow in Theoretical Physics

## Institute of Fundamental Sciences

**Auckland**

We seek a PhD graduate with some experience in the theoretical physics of quantum degenerate gases.

Closing date: 29 February 2008 Reference number: A058-08B

**For further information and to apply online, visit:**

**http://jobs.massey.ac.nz**

JP124333R

www.massey.ac.nz

Massey University
NEW ZEALAND

Te Kunenga ki Pūrehuroa

# University of Cologne

The **faculty of Mathematics and Natural Sciences of the University of Cologne** invites applications for

## 2 tenured professorships (W3 + W2) in Experimental Biophysics

Candidates are expected to have an excellent research background in **quantitive imaging or manipulation (eg. optical or mechanical) of biological systems on the molecular and cellular level.** The primary affiliation will be in the Physics Department, as part of the Bonn-Cologne Graduate School of Physics and Astronomy supported by the German Excellence Initiative. The candidates are expected to set up a strong research and training program in experimental biophysics, and to establish interdisciplinary research collaborations, in particular within the local Collaborative Research Centers (Sonderforschungsbereiche). The appointments are intended to further strengthen multi-disciplinary research activities encompassing the Natural and Medical Sciences at the University of Cologne, the local Max-Planck Institutes, and the Forschungszentrum Jülich.

The University of Cologne is an equal opportunity employer. Applications from women and disabled persons are particularly welcome.

Applicants are requested to send a summary of scientific development including a complete list of publications, a statement of research interests and a selection of maximally five reprints by **March 15th, 2008** to the **Dean of the Faculty of Mathematics and Natural Sciences, University of Cologne, Albertus-Magnus-Platz, D-50923 Köln**

W124395R

***Naturejobs* is pleased to present the**

# SPOTLIGHT ON MISSOURI

**DATE:**
MARCH 6, 2008

**DEADLINE FOR ADVERTISERS:**
FEBRUARY 28, 2008

Ranked 3rd in Percent of Patents in Biopharmaceuticals, Missouri is home to important and growing life science and biotechnology research. It is establishing itself as a leader in this industry by promoting collaborations among universities, research centers and companies across the entire state, from Kansas City to St. Louis. There are new additions and initiatives such as a new Life Science Center in Columbia and plans for an agricultural experiment station and research park. By demonstrating its commitment to research and development, Missouri is poised to grow in the life science and biotechnology industries.*

This Spotlight on Missouri offers a unique opportunity for the R&D community, research centers and companies within Missouri to gain international exposure in *Nature*, the world-renowned science journal. This Spotlight will be a valuable reference for readers interested in the region and will be eagerly read by potential employees, investors, decision-makers and influencers.

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www.cam.ac.uk/jobs/
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UNIVERSITY OF CAMBRIDGE

# University Lecturer

Department of Genetics
£33,779-£42,791 pa

Applications are invited for a University Lectureship in the Department of Genetics. Applications are encouraged from scientists who have demonstrated the potential to become leaders in their field of research. The Department particularly wishes to attract a candidate with an expertise in an area of quantitative, population or evolutionary genetics; however, applications from candidates in all areas of its broad research base in Genetics will be considered. The lecturer will be expected to interact with existing members of the Department in both research and teaching. In addition, the research environment of Cambridge offers many opportunities to establish collaborative links with members of other Departments and Faculties, including Medicine, Engineering and Physics. The successful candidate will be expected to play a full role in the teaching and research activities of the Department, including both the training of post-graduate students and post-doctoral Fellows, and in undergraduate teaching in the Faculty of Biology.

Appointments made at University Lecturer level will be for a probationary period of five years, with appointment to the retiring age thereafter.

**Further particulars and an application form may be obtained from http://www.gen.cam.ac.uk, or from the Secretary of the Department, University of Cambridge, Department of Genetics, Downing Street, Cambridge CB2 3EH (Email: t.oakley@gen.cam.ac.uk; telephone 01223 333987, fax 01223 333992). Applications should be sent to this address by no later than 29 February 2008 and should include a completed form PD18, a curriculum vitae, a list of publications, and a brief statement of research interests and future plans (no more than two pages).**

**Informal enquiries may be made to Dr David Summers (Email: dks11@cam.ac.uk).**

**Quote Reference: PC02974.**

**Closing Date: 29 February 2008.**

**The University is committed to Equality of Opportunity.**

U124528R

Andrea's Gift
Leading the fight against brain tumours in Yorkshire

Candlelighters

SCIENTIST TO LEAD BRAIN TUMOUR RESEARCH

**TWO YORKSHIRE CHARITIES, CANDLELIGHTERS AND ANDREA'S GIFT, HAVE JOINED FORCES AND ARE LOOKING FOR A RESEARCH LEADER TO ESTABLISH A BRAIN TUMOUR RESEARCH LABORATORY AT ST JAMES'S HOSPITAL, LEEDS**

Candlelighters is a successful Yorkshire based charity supporting research into children's cancer. Andrea's Gift is dedicated to funding brain tumour research in Yorkshire. Applications are sought from candidates with a strong track record in brain tumour research who have the ability to lead a team and a research programme.

There are close working relationships between the University and the Neuro-Oncology Services (Adult and Paediatric) and the Neurosurgical Department. The latter serves a population of 2.5 million for adult patients and nearly 4 million for paediatric patients. Funding will be available to the successful applicant for two years in the first instance, at a level commensurate with the proposed research and seniority of the applicant, and will be renewable for a further three years subject to favourable review of a project/programme grant application submitted within the first year of the appointment.

*Interested individuals are invited to submit a CV in confidence to* **Sally Amos** *at the* **Candlelighters Trust, Children's Day Hospital, St. James's University Hospital, Beckett St, Leeds LS9 7TF.**

*Informal enquiries would be welcomed by any of the following:* **Professor Alex Markham – 0113 206 5679; Professor Peter Selby – 0113 2064184; Dr Sue Picton – 0113 206 4985, Mr Paul Chumas – 0113 392 3297**

U124550RM

# Lost in today's ever-changing biosciences environment?

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*Naturejobs* and the Stanford School of Medicine have collaborated to bring you this video series featuring SoMCC "Industry Insights" and "Careers in Science" programs. This monthly series, delivered by top experts within the biomedical sciences and healthcare industries, will allow you to:

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- *How Should We Be Developing Drugs in the 21st Century?,* Hal Barron, MD, Genentech

**And stay tuned for these seminars coming soon:**

- *Intellectual Property Management & Technology Transfer,* Panel of Experts
- *Science & the Media,* Donald Kennedy, PhD, Emeritus Professor, Stanford
- *The Future of Personalized Medicine,* with Agilent Technologies

If you are interested to learn more about the SoMCC, please contact Suzanne Frasca, Program Coordinator, at (650) 725-7687 or somcareers@stanford.edu.

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THE UNIVERSITY OF WARWICK

Chemistry

# Assistant Professor - Experimental Materials Chemistry

£33,779-£40,335 pa **Ref: 30375-018**

*Warwick is one of Britain's leading universities with an enviable reputation for educational opportunities, first rate research and its commitment to the local community.*

*The University Values Diversity*

You will have the potential to have an internationally recognised and well-funded research group publishing in leading journals. You will have a strong reputation for research in any area of materials chemistry, including organic or inorganic materials, especially those relating to the high-technology fields of biomaterials, composites, electronic materials, catalysts, coatings and ceramics, and those complementary to existing strengths within the Department. A wide range of state-of-the-art equipment for such research is currently being established in the new £25M West Midlands Centre for Advanced Materials.

In addition to research excellence, you will demonstrate a commitment to teaching excellence at undergraduate and postgraduate levels and undertake administrative and pastoral duties normally associated with an assistant professorship at Warwick.

Informal enquiries to: Head of Department, Professor Peter Sadler FRS, P.J.Sadler@warwick.ac.uk; +44(0)24 7652 3818.

**Application packs are available from Personnel Services on 024 7652 3685 (24 hour answerphone), by email: recruit@warwick.ac.uk, our website below or www.jobs.ac.uk/warwick. An application form MUST be completed if you wish to apply for this post.**

**Closing date: 28 February 2008**

U124543R

**www.warwick.ac.uk/jobs**

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MAASTRO

GROW Research Institute Growth & Development

MAASTRO, Maastricht Radiation Oncology, is a co-operation between MAASTRO clinic, the University of Maastricht (UM) and the University Hospital Maastricht (azM) (see www.maastro.nl). MAASTRO consists of several division, including Maastro Clinic, which offers state-of-the-art radiotherapy to more than 3500 cancer patients each year from the Mid and South Limburg area in the Netherlands. MAASTRO clinic is also world-wide reference centre for Siemens Medical. In addition, research and training at Maastro is carried out in Maastro Physics, Maastro Trials, Maastro School, and Maastro Lab.

**MAASTRO Lab is a basic and translational research laboratory embedded within the GROW research institute of the Faculty of Health, Medicine and Life Sciences at Maastricht University. Research carried out in the past has been focused on the tumour microenvironment and EGFR signalling pathways, both of relevance to radiation oncology. MAASTRO Lab has made several important discoveries in these fields, including demonstration that EGFR is up regulated by radiation and that hypoxia inhibits the initiation step of mRNA translation. In addition, we have initiated translational and clinical studies based on these results including both phase I novel treatment and molecular imaging trials as well as a Biobank project with more then 1500 patients included.**

**The lab has 4 permanent scientists, 5 technicians, more then 5 PhD students and is fully equipped for cell culture, molecular biology, flow cytometry, hypoxia, gene expression, proteomics and microscopy. Maastro lab has set up the necessary infrastructure for controlled exposures to hypoxia and hypoxia/reoxygenation, including development of novel equipment that allows rapid and precise changes in oxygenation. Access to expertise, equipment and resources within the much larger GROW research institute and other facilities in the University are also readily available, including the genome centre, advanced microscopy, and the animal facility with its imaging facility (Optical imager, MRI 7Tesla and micro CTPET to come). MAASTRO has a structural collaboration with the VU in Amsterdam on molecular PET biomarkers, with the TU/Eindhoven on Systems Biology and is initiating a new collaboration with the University of Toronto on research related to the Unfolded Protein Response and tumour hypoxia. Maastro lab has a vacancy for a.**

# Senior scientist

## Head of Laboratory Research in molecular oncology (M/F)

Vac.nr. 2007.009/KC

**In this position you will be responsible** for carrying out basic and translational research that is of relevance to radiation oncology in the broadest possible scope. You will initiate an independent research program based on demonstrated skills and expertise in fundamental aspects of biology. In addition, you will be chiefly responsible for the scientific research and training within the lab of experimental Radiation Oncology (MAASTRO lab). As head of research you will manage the laboratory scientific research, direct the research policy, and participate actively in ongoing and newly initiated research lines and projects. Successful grant applications to prestigious (inter)national organizations to support expansion of research activities will constitute an important part of your work.

Depending on experience, the process to appoint you as professor or associate professor at the faculty of Health, Medicine and Life Sciences from Maastricht University will be started. You will participate in research and educational activities within the faculty. The emphasis in this faculty appointment is on microenvironment of solid tumours and cell signalling (EGFR) but there is room for your specific area of expertise.

**We are looking for a senior scientist** with training and experience in basic molecular biology, biochemistry, cell biology or related area. Candidates should have a proven track record or demonstrate a strong potential to function as a principal investigator, with high impact scientific publications and several large operating scientific grants. Candidates should have experience and knowledge of molecular oncology and have a recognized expertise within a specific research area relevant to radiation oncology. Experience in radiation biology, collaboration with clinicians and ability to speak Dutch is a plus but is not a prerequisite. Preferably candidates will have experience in research group management. In addition, candidates should be capable of formulating strategic goals for their research program in line with the organisational strategy.

**Conditions of Employment and salary** are based on the Dutch Collective Labour Agreement for Hospitals (CAO-Ziekenhuizen). You will receive a permanent contract on a fulltime basis (36 hours/week), depending on your relevant experience.

**Further information** will be gladly given by Prof. Philippe Lambin, head of the Dpt of Radiation Oncology azM (e-mail: philippe.lambin@maastro.nl) or telephone number: +31-(0)88-4455666. Please also visit www.maastro.nl and www.grow-um.nl.

**Your application letter, Curriculum Vitae and listing of publications** can be sent before the 28th of February 2008 to the department of Personell to the attention of mrs. T.Offermans, pbox 5800, 6202 AZ Maastricht, the Netherlands.

W124525R

## Forschungszentrum Karlsruhe
in der Helmholtz-Gemeinschaft

**The Institute of Toxicology and Genetics (ITG) is looking for outstanding candidates for a**

### Junior group leader position

The Institute of Toxicology and Genetics (ITG) is a dynamic and excellently equipped biomedical research unit located within the campus of the Forschungszentrum Karlsruhe. Current research focuses on a variety of biomedical topics, including molecular toxicology, cancer, development, disease-related changes in cell signalling and animal models of human diseases with zebrafish and mouse as the main animal systems.

The new **NanoBiology** research program **aims to investigate molecular and cellular interactions at functional interfaces,** including cell membranes, cell substrates and the surfaces of macromolecular assemblies. Interactions at such interfaces are pivotal for the control of key cellular processes. Manipulating these interactions both in cells and organisms is key to our understanding of these processes, and also for the development of novel technologies aimed at functionally modulating them.

Within the context of the NanoBiology research program, applications are invited from candidates who use interdisciplinary approaches to investigate the biology of stem cells (including cancer stem cells) and/or to develop novel sensors and detection devices to investigate cellular function. Experimental projects should focus on methods commonly used in systems biology, chemical biology, synthetic biology and/or combine approaches in physics, chemistry, microsystems engineering or nanotechnology to address questions of cell behaviour and differentiation in an innovative manner. The main criterion for selection will be an excellent research record and research interests that complement existing research topics. Details about existing research topics are available upon request.

We strive to employ equivalent numbers of men and women in the work place, and therefore especially encourage women to apply.

Informal enquiries can be made to Prof. Dr. Uwe Strähle, phone: +49-7247-82-3291, email: uwe.straehle@itg.fzk.de, Prof. Dr. Jochen Wittbrodt, phone +49-7247-82-3703, email: jochen.wittbrodt@itg.fzk.de, or Prof. Dr. Jonathan Sleeman, phone: +49-7247-82-3291, email: sleeman@itg.fzk.de.

Interested candidates are invited to apply online on our homepage (->Job/Training) within the next four weeks or to send copies of degree certificates, a CV, a list of publications (with an indication which are considered to be the best five), a brief summary of intended future research and the names and addresses of two or three referees quoting the vacancy number **541/2007** to:

**Forschungszentrum Karlsruhe GmbH**
**Attention Mr. Speck Personnel Department**
**P.O. Box 3640, D-76021 Karlsruhe, Germany**

**Internet: www.fzk.de**

W124534R

## Three Ph.D. grants available in Meteorology in Copenhagen, DK

Three Ph.D. student positions in atmospheric modelling are available at the Danish Meteorological Institute and the Niels Bohr Institute, University of Copenhagen. Two of the positions will be in the field of "on-line coupled" air pollution modelling as part of "Centre of Energy, Environment and Health" (see further information here: **http://ceeh.dk/English/index.html**) and one within atmospheric physics modelling as part of the project "Solar/electric heating systems in the future energy system" (see **http://dmi.dk/eng/index/job.htm**).

The successful candidate should hold a master degree in Geophysics/ Physics, Meteorology, Environmental Sciences, Chemistry or Mathematical Modelling. Experience within geophysical fluid dynamic modelling/methods/programming is an advantage.

*The deadline for application for all three positions is* **15 March 2008** *and the positions should be occupied as soon as possible thereafter.*

W124334RM



## School of Medical Sciences
# Research Fellow

An ambitious and bright postdoctoral research fellow, you will study the dynamic regulation of amino acid starvation responses in yeasts. This collaborative, interdisciplinary project, led by Al Brown and George Coghill, integrates mathematical modelling with experimental biology. You will join the internationally renowned Aberdeen Fungal Group.

You should hold a PhD in biochemistry, molecular biology, microbiology, or a related discipline. Postdoctoral experience in fungal cell and molecular biology, and expertise in genomics or biochemistry will be an advantage.

This BBSRC-funded position is available for three years in the first instance.

Salary will be at the appropriate point on the Grade 6 or 7 scale (£27,857 – £40,909 per annum), with placement according to qualifications and experience.

Informal enquiries to Professor Al Brown (e-mail: al.brown@abdn.ac.uk).

Online application forms and further particulars are available from www.abdn.ac.uk/jobs. Alternatively, telephone (01224) 272727 (24-hour answering service) quoting ref: YMB148R an application pack.

Closing date: 7 March 2008.

Promoting Diversity and Equal Opportunities throughout the University

1 4 9 5 UNIVERSITY OF ABERDEEN

U124586R

## PROFESSOR POSITION

*available in the*
***DEPARTMENT OF PHYSIOLOGY, BIOPHYSICS AND NEUROSCIENCE***
*of the*
***CENTER OF RESEARCH AND ADVANCED STUDIES (CINVESTAV) IN MEXICO CITY***

for scientists interested in working of research mentioned in our web page:

**http/www.fisio.cinvetav.mx/ investigación/index.html**

Candidates must fulfil the following requirements:

1) PhD degree in any area of Biomedical Sciences.
2) Two years (as minimum) postdoctoral experience at a prestigious Institution.
3) At least six publications in prestigious journals.

Candidates should submit before 29th February 2008 a letter of application and CV to:

**Mrs. Yolanda Ávila**
**CINVESTAV**
**Ave. IPN 2508, Mexico, D.F 07360**
**Phone (5255) 5061 3967**
**Email:**
**yavila@fisio.cinvestav.mx**

The selected candidates should provide before 28th March 2008, three referenced letters by well-known investigators in their field, a working project, give a seminar of their current work to department faculty and sustain interviews with selection committee.

NW124012R

## Director of the Brook Byers Institute of Sustainable Systems, Georgia Tech

Georgia Tech is seeking an innovative and dynamic Director of the newly formed Brook Byers Institute for Sustainable Systems **http://sustainability.gatech.edu** The Director will hold the position of Hightower Chair for Environmental Technologies and Georgia Research Alliance Eminent Scholar. Applications should be submitted online at **http://www.ce.gatech.edu/jobs/**

*Requests for information should be directed to* **Judith Curry** *at* **curryja@eas.gatech.edu 404. 894. 3948**; *or* **Joseph Hughes** *at* **joseph.hughes@ce.gatech.edu 404. 894. 2201.**

*Georgia Tech is an Equal Opportunity Employer*

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**Novo Nordisk**
**R&D – Pre-Clinical Development**
**Research Scientist**

For the immunogenicity assessment in preclinical and clinical studies. We are seeking an experienced and motivated immunologist to the Antibody Analysis department with exhaustive experience within the function of the immunesystem in general and the humoral immuneresponse in particular. Ref: "NN38521 Research Scientist". Deadline: 18 Feb 2008.

**Contact**

Lisbeth Bjerring Jensen
Tel: +45 44 42 6849
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KU THE UNIVERSITY OF KANSAS

## HOWARD E. MOSSBERG DISTINGUISHED PROFESSOR OF PHARMACOGENOMICS:

### PHARMACOLOGY/TOXICOLOGY & HIGUCHI BIOSCIENCES CENTER

Applications are invited for appointment as a **tenured, Distinguished Professor at the Full Professor level** in the Department of Pharmacology & Toxicology and the Higuchi Biosciences Center (HBC) at the University of Kansas. The research focus of the Department of Pharmacology and Toxicology is in neuropharmacology and neurotoxicology. The HBC is a multidisciplinary research and technology development center in biomedical and pharmaceutical sciences. The HBC and Department have programs focused on drug target discovery, drug design and delivery, high throughput screening, genomics, proteomics, and transgenics/knockout animal models. We are looking for an individual with a strong research program in the areas of genomics or genetics, preferably related to pharmacological/toxicological or neuroscience research. The successful candidate must hold a Ph.D., MD, or DVM, have a strong record of externally funded research, and previous teaching experience at the undergraduate and/or graduate levels. The person appointed to this position is expected to participate in or lead collaborative research projects. Excellent core facilities exist including those for genomics, DNA sequencing, protein analysis, peptide synthesis, fermentation, cell culture, confocal/electron microscopy and imaging, molecular modeling, NMR, mass spectrometry, X-ray crystallography, and MRI. To apply, send curriculum vitae, a description of research plans, and the names of 3 references to: **Dr. Elias Michaelis, Higuchi Biosciences Center, 2099 Constant Ave., University of Kansas, Lawrence, KS 66047; e-mail: emichaelis@ku.edu**. Review of applications begins March 1, 2008, and will continue until the position is filled.

*The University of Kansas is an Equal Opportunity Employer.*
*Under-represented minorities and women are encouraged to apply.*

NW124195R

Je veux

## M'investir

L'Université de Sherbrooke propose un milieu de travail exceptionnel pour combler vos désirs de dépassement.

**PROFESSEURE OU PROFESSEUR EN MICROBIOLOGIE MOLÉCULAIRE**
**Faculté des sciences**
**Offre nº 08-6-08-04**

Les curriculum vitæ doivent être reçus avant 17 h, le vendredi 14 mars 2008.

Visitez notre site pour connaître la description complète de ce poste et les modalités pour poser votre candidature.
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*L'Université de Sherbrooke souscrit à un programme d'accès à l'égalité en emploi et un programme d'équité en emploi pour les femmes, les membres des minorités visibles et ethniques, les Autochtones et les personnes handicapées.*

NW124299R

UNIVERSITÉ DE SHERBROOKE

## Stanford Molecular Imaging Scholars (SMIS) Program

**Postdoctoral Training in Molecular Imaging of Cancer**
**Stanford University, Stanford, CA, USA**
***Principal Investigator*: Sanjiv Sam Gambhir, MD, PhD**

The Stanford Molecular Imaging Scholars (SMIS) Program is a diverse training program bringing together more than thirteen Departments, predominantly from the Stanford Schools of Medicine and Engineering, in order to train the next generation of interdisciplinary leaders in molecular imaging. Oncologic molecular imaging is a rapidly growing area within molecular imaging which combines the disciplines of chemistry, cell/molecular biology, molecular pharmacology, bioengineering, imaging sciences, and clinical medicine to advance cancer research, diagnosis, and management. SMIS fellows will conduct innovative research in cancer imaging under the supervision of two faculty mentors from complementary fields, in a comprehensive, integrated, flexible program (up to 3 years). Funding is available for stipend, supplies, and travel.

- Application deadline: **May 12, 2008** for a start date in September, 2008.
- Applicants must have a PhD or MD and must be US citizens or permanent residents.
- For more information: **http://mips.stanford.edu/smis/**
- **Inquiries to Sofia Gonzales (650) 724-9139; sofias@stanford.edu**

NW122945R

## Department of Pharmacology
## University of Minnesota Medical School

## TENURE/TENURE TRACK POSITION

### (Assistant Professor, Associate Professor, Professor)

The Department of Pharmacology at the University of Minnesota invites applications for a tenure/tenure track faculty position at the rank of Assistant Professor, Associate Professor or Professor. The successful candidate will be expected to develop an innovative, competitive research program supported by extramural funding and to participate in teaching undergraduate, graduate and professional courses. Applicants using molecular, biochemical, cellular or integrative approaches to study problems relevant to pharmacological sciences are encouraged to apply. Requirements for the Assistant Professor position include a Ph.D. in Pharmacology or other basic biomedical science, and/or an M.D. degree, and at least three years of relevant postdoctoral research experience. Applicants must have a strong record of research accomplishments, as documented by publications in leading peer-reviewed journals. Associate Professor or Professor applicants must have professional distinction in published research, teaching and evidence of consistent extramural funding for research.

For additional information on our department, visit our Website at: **www.pharmacology.med.umn.edu**

*Interested applicants should apply online at* **https://employment.umn.edu** *for either requisition 153317 (Assistant Professor) or 153318 (Associate Professor or Professor), and attach a letter of interest, curriculum vitae, brief statement of research interests and names of three references.*

NW123700R

nature methods

## Locum Assistant Editor

*Nature Methods* seeks a Locum Assistant Editor to join their editorial team for a period of six months to cover a maternity leave. The journal publishes high quality papers that represent major methodological developments, likely to be influential in the life sciences. In the tradition of *Nature* journals, this selection relies on a thorough peer review process.

For more information about the journal, see our website (**http://www.nature.com/nmeth**).

Members of the editorial team evaluate manuscripts, oversee the peer review process, commission and edit secondary materials such as Reviews, and write short pieces and editorials for the journal. The new editor will join our team in the NYC office of the larger Nature Publishing Group.

Candidates should have a broad interest in science, excellent communication skills, and a willingness and ability to learn new fields. Applicants should have completed a Ph.D. in any of the areas covered in *Nature Methods*.

To apply, please submit a CV, and a cover letter explaining your interest in the position and your possible start date to Human Resources Department, Nature Publishing Group, e-mail: **admin@natureny.com**

Applications should arrive as soon as possible with a *close date of February 28, 2008*.

NPG is an EOE.

nature publishing group npg

IN123698R

## FACULTY POSITION IN CARDIOVASCULAR BIOCHEMISTRY

### DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

### SAINT LOUIS UNIVERSITY SCHOOL OF MEDICINE

Saint Louis University, a Catholic Jesuit institution dedicated to student learning, research, health care, and service is seeking applicants for a faculty position in the Edward A. Doisy Department of Biochemistry and Molecular Biology for a tenure-track position involving research and teaching at the ASSISTANT PROFESSOR level, although outstanding candidates at a more senior level will be considered. The department is housed in the E.A. Doisy Research Center, a brand new state-of-the-art facility designed to foster collaboration between investigators. We seek applicants who use innovative approaches in the areas of cardiovascular biology, metabolism and signaling. Cardiovascular research is a growing focus area at the School of Medicine with strengths in lipidomics, cell signaling, systems biology and molecular basis of cardiovascular disease. We are interested in a highly interactive candidate whose interests will complement ongoing research programs in the cardiovascular sciences in the Department as well as the University.

The successful candidate is expected to establish a strong extramurally-funded research program. A potential for or demonstrated evidence of competing successfully for external funding will be important criterion for selection. Excellent start-up funds and salary are available. Interested candidates must submit a cover letter, application and current curriculum vitae to http://jobs.slu.edu. Additionally send current curriculum vitae, description of research plans, and at least three letters of recommendation to:

Search Committee
c/o William S. Sly, M.D., Chairman
Edward A. Doisy Department of
Biochemistry and Molecular Biology
Saint Louis University School of Medicine
1100 South Grand Blvd.
St. Louis, MO 63104-1028

*Saint Louis University is an Affirmative Action, Equal Opportunity Employer, and encourages nominations and applications of women and minorities.*

NW123553R

*The* **UNIVERSITY** *of* **VERMONT**

Molecular Physiology & Biophysics

## FACULTY POSITION

The Department of Molecular Physiology & Biophysics at the University of Vermont is seeking to recruit a **Cell Biologist/Biophysicist** at the **Assistant Professor** level on the tenure-track, although Associate and Full Professor candidates will be considered.

The Department has significant strength in protein structure and function with emphasis on contractile and cytoskeletal proteins. The ideal candidate will complement existing expertise in molecular biology, single molecule biophysics, and structural biology. The candidate will be expected to develop an independent, extramurally funded research program in cell biology with emphasis on ***mechanisms by which the cytoskeleton and molecular motors govern cellular function*** (e.g. cell signaling, intracellular transport, and cell division) which may be altered in human cancer and cardiovascular diseases.

The candidate must be willing to team-teach mammalian physiology in a medical school setting. Start-up funds will be competitive, and access provided to graduate students and postdoctoral fellows through departmental training grants.

Review of applications will begin immediately, and continue until the position is filled. Include a résumé, research plan, teaching experience, and the names of three references whose letters must be received prior to review of your application.

*Address all inquiries and materials to*
**Dr. Robert Low**
**Chair Faculty Search Committee**
**Department of Molecular Physiology & Biophysics**
**Health Science Research Facility**
**149 Beaumont Avenue**
**Burlington, VT 05405-0075 U.S.A.**
**Bob.Low@uvm.edu**

Or apply online at
**www.uvmjobs.com**

*The University of Vermont is an equal opportunity/affirmative action employer. Applications from women and people from diverse racial, ethnic and cultural backgrounds are encouraged.*

NW124419R

The University of Massachusetts Medical School (UMMS)

*is seeking a*

# CHIEF SCIENTIST

to direct a newly established International Stem Cell Registry and Human Embryonic Stem Cell Bank. Reporting to the Stem Cell Center Director, this tenure track position and program will be housed in a state-of-the-art laboratory and administrative facility. This is an excellent opportunity for an outstanding scientist to provide leadership for a staff of over 20 technical and support personnel and grow the program with the flexibility to pursue extramurally funded human embryonic stem cell research.

The stem cell program will work closely with new RNAi Therapeutics and Gene Therapy programs. These three programs are a vital component of UMMS commitment to clinical and translational science. The program will also play a pivotal role in the development of UMMS Advanced Therapeutic Cluster (ATC), a key element of the Commonwealth of Massachusetts Life Science Initiative.

Applicants must have a PhD, MD/PhD, or MD degree (or equivalent), accomplishments in biomedical research, and have a track record of administrative expertise, leadership, and research in scientific areas relevant to stem cell biology.

As an equal opportunity and affirmative action employer, UMMS recognizes the power of a diverse community and encourages applications from individuals with varied experiences, perspectives, and backgrounds.

**Applicants should send a curriculum vitae and cover letter electronically, briefly outlining their interest in the position to:**

**gary.stein@umassmed.edu**

**Dr. Gary S. Stein**
**Chair, Search Committee**
**Department of Cell Biology**
**UNIVERSITY OF MASSACHUSETTS MEDICAL SCHOOL**
**55 Lake Ave. North Worcester, MA 01655**

NW124594R

**www.cam.ac.uk/jobs/**
A world of opportunities

UNIVERSITY OF CAMBRIDGE

## Electron Microscopy Manager

Department of Physics
Salary: £30,012 - £40,335 pa

The Department intends to appoint a Manager to be responsible for the management and development of the Electron Microscopy facility, which comprises one transmission electron microscope (TEM), one scanning electron microscope (SEM), two environmental SEMs (ESEM) and a state of the art dual beam instrument (ESEM/FIB), all housed within a single suite of rooms. The facility supports the work of a number of research groups as well as providing a service to other Departments in the University. The Manager will also have an opportunity to run their own research programme, including the supervision of PhD students.

It is expected that the successful candidate will have a PhD (or postgraduate qualifications and experience) in a relevant scientific discipline, plus wide experience and knowledge and experience of electron microscope techniques. A basic understanding of high-vacuum methods, high-voltages, electronics, computers and system networks is essential. Appropriate management and interpersonal skills will also be expected.

This is a permanent appointment subject to the successful completion of a probationary period.

**Further details of the post may be obtained from the Academic Secretary and also from the Department's website (http://www.phy.cam.ac.uk).**

**Applications, including a curriculum vitae and a completed form PD18 (Parts I and III only), available with the further particulars, or at http://www.admin.cam.ac.uk/offices/personnel/forms/pd18/ should be sent to the Academic Secretary of the Department, Department of Physics, The Cavendish Laboratory, JJ Thomson Avenue, Cambridge CB3 0HE.**

**Please quote reference: KA02874.**

**Closing date: 29 February 2008.**

**The University is committed to Equality of Opportunity.**

U124454R


## Career articles from Naturejobs

- **Naturejobs Prospect**: quick takes on career implications of current events
- **Naturejobs Special Report**: examinations of jobs issues on both sides of the bench
- **Naturejobs Careers & Recruitment**: discipline-by-discipline exploration of opportunities
- **Naturejobs Regions**: tours of scientific hubs
- **Naturejobs Movers**: traffic reports that follow high-profile scientific globe-trotters and sector-hoppers
- **Naturejobs Postdocs & Students**: guides to taking a step toward a permanent position

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**Executive Director**
77-A, St.45, F-10/4, Islamabad, Pakistan

RW124692R

At the **Faculty of Medicine, Institute of Anatomy**, the position of a

## W3-Professor of Anatomy (Chair II)

**(Succession of Prof. Dr. med. E. Lütjen-Drecoll)**

is available by 1st April 2010.

The successful candidate will represent the discipline in research and teaching. Applicants should have broad teaching experience in all aspects of Anatomy. At present, Chair II has a focus on neuroscience and glaucoma research. Cooperation with other research groups of the Faculty of Medicine such as DFG Collaborative Research Centers and the Graduate Schools are expected. The Faculty of Medicine offers teaching programs in Medicine and Dentistry and a bachelor/master program in Molecular Medicine.

The candidate must have academic qualifications at the level of Ph.D. or M.D. degree, teaching skills, and professional experience, preferably but not necessarily in a junior faculty position. Moreover, experience as a group-leader, excellent publications, and above-average fund-raising are expected.

Applicants must not be older than 52 years at the time of appointment. Exceptions are possible but depend on the consent of the Bavarian Ministry of Science, Research, and Art and the Bavarian Ministry of Finances (Art. 10 Abs. 3 Satz 2 BayHSchPG).

The University of Erlangen-Nuremberg intends to increase the number of women in research and teaching positions and, therefore, strongly encourages female researchers to apply. Disabled applicants will be preferentially considered in case of equivalent qualification.

Please send a letter of application, a resume (picture required), a structured list of publications and teaching activities (one copy in written form, one copy on data CD) as well as officially certified copies of credentials and certificates to the Office of the Dean of the Medical Faculty of the University of Erlangen-Nuremberg, Oestliche Stadtmauerstrasse 30a, 91054 Erlangen, Germany. The deadline for application is the **20th March 2008**.

**Friedrich-Alexander-University Erlangen-Nuremberg**

**www.uni-erlangen.de**

W124538R

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REGION MIDI-PYRENEES
HOTEL DE REGION
22 AVENUE DU MARECHAL JUIN
31406 TOULOUSE CEDEX 9

## INTERNATIONAL CHAIRS PIERRE DE FERMAT MIDI-PYRENEES

The Conseil Régional Midi-Pyrénées has decided to establish the Pierre de Fermat Highly Qualified Chairs to welcome foreign professors and researchers, in any discipline, with an international recognized reputation.

Each chair allows the foreign scientist to be hosted for 6 or 12 full months in research institutions in Midi-Pyrénées.

The global financial amount attributed to each project can go up to €56,000 for a 6 months chair or €112,000 for a 12 months chair.

An interdisciplinary and international jury will select the candidates every year based on quality criteria. The application is presented by the host group to the Jury.

The deadline for the application for the present call is june 16, 2008

**Contact Direction de l'Action, Economique et de la Recherche, Service RECHERCHE Tél : + 33 (0)5 61 33 57 18**
**www recherche.midipyrenees.fr www midipyrenees.fr**

W124291A

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"Through our posting for a post-doc position at NatureJobs we have received messages from a surprisingly large number of highly qualified investigators from all over the world, and have been able to recruit a suitable candidate. Thank you very much."

Alberto Sánchez-Fueyo, MD,
Hospital Clínic Barcelona/IDIBAPS, Spain

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DIABETES RESEARCH WELLNESS FOUNDATION

## DRWF Open Funding 2009

**The Diabetes Research and Wellness Foundation invites the submission of research projects and proposals requiring funding in the field of diabetes.**

Full parameters for applications may be obtained from the charity or its website.

Types of application currently considered suitable (with a budget of up to £30,000) would be:

- **Research Projects** – clinical or non-clinical, of one year's duration (though extensions may be considered);
- **Exchange Fellowships** (especially between the UK and the USA).

Applications must be received on or before Friday, 6 June 2008. The charity's Research Advisory Board meets in October. Applicants will be notified early November and, if successful, expected to take up the grants early in 2009.

Applications should be a maximum 4 sides A4, single line spaced with an 11 or 12 point, clearly readable, font. They should include (as appropriate):

*Page 1*

- Applicant's name, qualifications, present post and contact details;
- Name and address of the Institution(s) where the work will be carried out, Head of Department/Institution and major participants in the project;
- Signed verification of funding application by Head of Department and department/ institution authorities stating "I confirm that this application has been read and that, if granted, the work will be accommodated and administered in the department/ institution".

*Page 2*

- Outline of the proposed research comprising Title, Research question, Relevance to diabetes.

*Page 3*

- Lay summary, Methodology, Expected outcome.

*Page 4*

- Any additional information to support the application (references can be included);
- Amount requested, with a general breakdown of costs.

PLUS: Separate single sheet of A4: (Brief) Curriculum Vitae of main applicant

11 (eleven) hardcopies are required and an electronic copy should be sent as a single word document.

*Applications or enquiries should be directed to:*
**Open Funding Programme**
**Diabetes Research and Wellness Foundation,**
**Office 101-102, Northney Marina, Hayling Island, Hants. PO11 0NH**
**Tel: 023 926 36135**
**Email: research@drwf.org.uk**

U124060A

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Fundación **BBVA**

# 2008 **BBVA** Foundation
# Frontiers of Knowledge Awards

The mission of the BBVA Foundation is to promote scientific research of excellence in a range of knowledge areas and to support creative activities in the arts, principally literature, music and fine arts. The Foundation's work expresses the commitment of financial group BBVA to building a better future for people.

The **BBVA Foundation Frontiers of Knowledge Awards** seek to recognize research in the basic sciences, biomedicine, the environment, information technologies and economics, along with creative output in the arts. Awards are also granted for research and/or projects addressing two core concerns of the 21st century, climate change and development cooperation.

| The BBVA Foundation Frontiers of Knowledge Awards cover the following categories: |
|---|
| ■ Basic Sciences (Physics, Chemistry, Mathematics) |
| ■ Biomedicine |
| ■ Ecology and Conservation Biology |
| ■ Information and Communication Technologies |
| ■ Economics, Finance and Management |
| ■ Arts (Music, Painting, Sculpture, Architecture) |
| ■ Climate Change |
| ■ Development Cooperation |

The **BBVA Foundation Frontiers of Knowledge Awards** will consist of €400,000, a diploma and commemorative artwork for each prize category.

Candidates may be one or more natural persons, without limit of number, of any nationality. This means recognition may go to achievements resulting from cooperation within or between teams. In the categories of Climate Change and Development Cooperation, entries are also open to public or private non-profit organizations.

Nominations can be made by the following institutions: scientific or artistic societies and organizations; national or regional academies of the sciences or the arts; public or private R&D centers; university departments and schools and university or research institutes; hospital departments and biomedical research centers; conservatories and schools of music; museums of the arts and sciences; and public agencies and other organizations substantially engaged in analysis and/or activities relating to climate change and development cooperation.

Candidate selection will be guided by the principles of merit and objectivity and will rely on the best standards and metrics of excellence in each prize area. The Foundation will be partnered in the selection process by the Consejo Superior de Investigaciones Científicas (CSIC), Spain's premier multidisciplinary public research organization. Distinguished international experts will be appointed as members of the prize jury in each category.

Entries can be submitted from January 2, 2008 to June 30, 2008 using the online form provided on the dedicated website: www.fbbva.es/awards.

With the collaboration of:

CSIC CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

W124062A

Fondation IPSEN, *Nature Medicine* and *Nature Immunology* present:

# An Emergence & Convergence mini-symposium
# Multiple Sclerosis: From Pathogenesis to Therapy

Multiple sclerosis is an inflammatory autoimmune disease targeting the central nervous system, leading to demyelination and axon degeneration and to severe disability as the disease progresses. Multiple sclerosis presents as a clinically heterogeneous disease, which has been problematic for efforts to develop appropriate animal models. Many environmental and genetic factors have been identified that may initiate disease. Various immune and neural cells have been found to play key roles in disease pathogenesis and progression. This Emergence & Convergence mini-symposium will address open questions in multiple sclerosis research, with the goal of identifying future directions that may lead to therapy.

**June 6, 2008**
**Espace Charles-Louis-Havas,**
**Paris, France**

**CHAIR**
**Jean-François Bach**
(Hôpital Necker, France)

**SPEAKERS**
**Burkhard Becher**
(Univeristy of Zurich, Switzerland)
**Christian Confavreux**
(Hôpital Neurologique Pierre Wertheimer, France)
**Britta Engelhardt**
(University of Bern, Switzerland)
**Vijay Kuchroo**
(Harvard Medical School, USA)
**Roland Martin**
(Center for Molecular Neurobiology - Hamburg, Germany)
**Stephen Sawcer**
(University of Cambridge, UK)
**Kenneth J. Smith**
(University College London, UK)
**Larry Steinman**
(Stanford University, USA)

**ORGANIZERS:**
**Eva Chmielnicki**
(*Nature Medicine*, USA)
**Laurie Dempsey**
(*Nature Immunology*, USA)
**Yves Christen**
(Fondation IPSEN, France)

**Application and Abstract Submission deadline:**
**March 31, 2008**

Electron microscope image of demyelination in the lumbar spinal cord of a mouse after experimental autoimmune encephalomyelitis. Image courtesy of Wutian Wu. Image design by Katie Vicari.

Attendance at this meeting is free on acceptance of application.
To apply and for more information visit **www.nature.com/natureconferences/eandc/MS**

a nature conference

FONDATION IPSEN | nature medicine | nature immunology

IN122209E

CANCER RESEARCH UK

# Beatson International Cancer Conference

Co-sponsor ASSOCIATION FOR INTERNATIONAL CANCER RESEARCH

## Cell Growth, Metabolism and Cancer

*Sunday June 15 to Wednesday June 18 2008 Glasgow, Scotland*

**Speakers and Sessions**

**Keynote Address:** William Kaelin (US), Craig Thompson (US)
**Cell Growth I:** Michael Hall (CH), Angus Lamond (UK), Peter Vogt (US), Jonathan Warner (US)
**Cell Growth II:** Bruce Edgar (US), Robert Eisenman (US), Robert White (UK)
**Cell Death and Survival I:** Beth Levine (US), Scott Lowe (US), Kevin Ryan (UK), Eileen White (US)
**Cell Death and Survival II:** Doug Green (US), Nissim Hay (US), Marja Jaattela (DK), Adi Kimchi (IL), Guido Kroemer (FR)
**Tumour Cell Metabolism:** Dario Alessi (UK), Chi Dang (US), Eyal Gottlieb (UK), Peng Huang (US), Sally Kornbluth (US), Reuben Shaw (US)
**Tumour Microenvironment:** Adrian Harris (UK), Peter Ratcliffe (UK), Gregg Semenza (US), Celeste Simon (US)

**Aims of the conference**

The metabolic traits of cancer cells differ from their normal counterparts. The understanding of the mechanisms that alter growth and metabolism in cancer cells will be discussed. It is well established that molecular signalling links the metabolic pathways of cells, with cues for both cell growth and cell death. Although it is still unclear whether metabolic changes play a causal or supportive role in tumourigenesis, the special metabolic demands of cancer cells provide a target for therapy.

Short talks will be granted to the authors of outstanding abstracts. Some financial assistance will be available to presenters of these short talks through sponsorship from the Association for International Cancer Research. For additional information, registration forms and details for poster presentation please contact:

Tricia Wheeler, Conference Co-ordinator, Beatson Institute for Cancer Research, Garscube Estate,
Switchback Road, Bearsden, Glasgow G61 1BD, UK
Tel: (24 hrs) +44 (0) 141 942 0855 Fax: +44 (0) 141 330 6426 email: t.wheeler@beatson.gla.ac.uk
Website, on-line registration and abstract submission: http://www.beatson.gla.ac.uk/conf

**Deadline for registration, payment and abstract submission: April 11, 2008**

the Beatson institute for cancer research

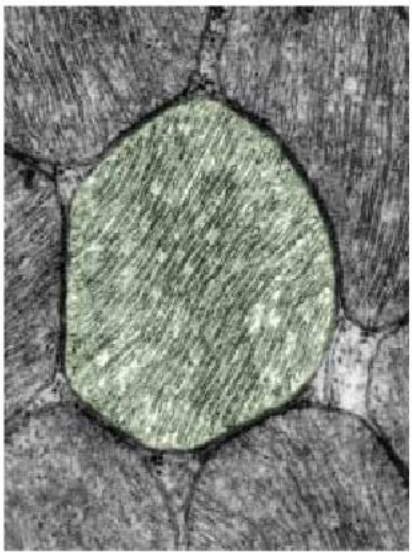
AICR FUNDING THE FIGHT AGAINST CANCER

CANCER RESEARCH UK

U120697E

# The seventh European Meeting on Mitochondrial Pathology

## *-From basic mechanisms to disease and ageing*



## June 11-14, 2008, Stockholm, Sweden

Registration is now open!

**Confirmed speakers:**

Vera Bianchi, Dan Bogenhagen, David Chan,
Zofia Chrzanowska-Lightowlers, Salvatore DiMauro,
Jose A. Enriquez, Maria Falkenberg, Claes M. Gustafsson,
Jun-ichi Hayashi, Ian Holt, Howard T. Jacobs, Dongchon Kang,
Nils-Göran Larsson, Kari Majamaa, Jodi Nunnari, Anders Oldfors,
Pierre Rustin, Elena Rugarli, Ann Saada, Eric Shoubridge,
Jan Smeitink, Anu Suomalainen, Aleksandra Trifunovic,
Douglass Turnbull, Douglas C. Wallace, Massimo Zeviani

All participants are invited to present posters. Posters will be selected for oral presentations based on submitted abstracts. Registration closes at May 1st 2008.

W123909E

## All details can be found at *www.mitomed.se/euromit7*

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NATURE|Vol 451|7 February 2008

# Chess's game

It's a grey area.

Nye Joell Hardy

Ian Rodriguez rushed past his staff who were crawling across the labs' floors, looking under tables and lab equipment. He glared at a retinal scanner, which permitted him access to Lab 5.

Nothing had changed.

In the first glass enclosure, eight-week old tobianos — all fifth-generation clones — kitten-staggered through their food dishes. Each kitten was black and white. Specifically, their left sides were black and their right sides were white.

The next enclosure contained eight overos: three-month-old, second-generation clones. The kittens wrestled one another, ignoring him. Their vertical black and white stripes reminded Ian of old-fashioned prison uniforms.

The last enclosure belonged to Chess. Its glass door was agape. The cage was empty.

Each enclosure had an access panel, two-way retinal scanners and video surveillance. How did a thief get out of the lab unnoticed? Ian punched numbers into an intercom. "Dr Rodriguez here. I'm with the cancer cats. Pull video for GP Lab 5, Section 3, and send the feed to my office. The last 12 hours."

The cats were clones of an original 'foundation' cat, Sammy, but Ian's gene-insertion techniques controlled their coat colours. When Texas A&M had started the cat-cloning business 20 years earlier, they could not have known what they had stumbled upon: mammalian blood vessels grew in random patterns, even in clones. In feline embryos, the blood's heat activated certain proteins in its fur, darkening it. If the vessels were close to the skin, the fur was black in that location. If the vessels were not, the fur remained white. This meant that a cat's coat colour showed where the blood vessels grew under the skin. Ian's cats' perfect patterns showed that his gene-insertions could control previously unpredictable vessel growth.

Cancer cells also grew in random patterns, just like blood vessels. Ian's gene therapies could be adapted to stop cancer growth, so Chess was the most important and expensive cat he'd ever made.

Ian returned to his office and reviewed the security footage. When he got to Chess's debut, he watched it twice: the door fell open by itself. Chess jumped down to the floor.

"A door malfunction?"

The cameras tracked only the activity of taller humans, so Chess was invisible on the floor. He was probably in the building, but no one knew where. Ian needed aspirin.

In the lunchroom, he could barely stand the laughter as he jammed coins into a vending machine.

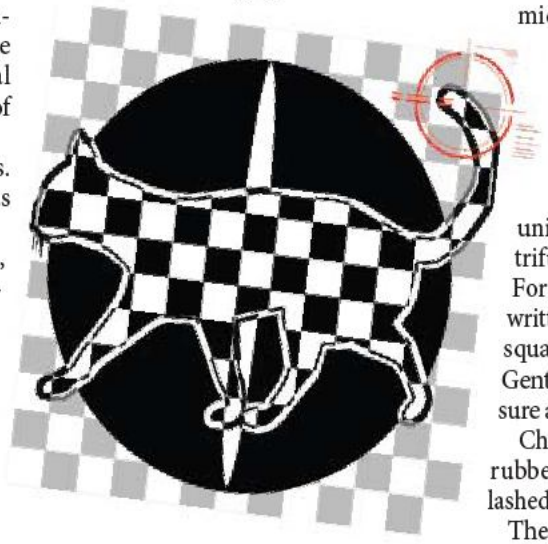
"The world's not black and white, you know!"

"Sure it is! Zebras, pandas, orcas…"

"… soccer balls…"

"Soccer balls aren't alive, you idiot."

Ian pulled two aspirins out of the machine and gulped them down without water. Then an unearthly screech made him choke.



JACEY

Everyone was staring at the longest wall in the cafeteria. Again a wavering cry. Ian recognized the offended yowl of a Siamese cat.

His cat had escaped through a floor vent, climbed up into the duct work and then fallen through the wall. To the surprise of everyone in the lunchroom, Ian started kicking at the wall until he'd made a ragged hole.

Mid-wail, Chess fell silent. Worried, Ian backed away, waiting and listening.

A triangular head poked out from the wall. As the cat pulled out its lithe body, everyone fell silent.

Whiskers to fur to nails, a black-and-white checkerboard blanketed the cat. Each square was 1.86 centimetres to a side. With pupils so dilated with rage that they eclipsed the bright blue irises, ears flat back against his head, and his long tail twitching a furious semaphore, Chess fast-slunk towards the corner. His short fur audibly brushed the wall.

Ian grabbed a lidded wastepaper basket, dumped the trash, and gave chase. As the cat reached the corner, Ian scooped him up, quickly righted the can, and replaced the lid.

Standing up, he grinned at the surprised employees.

A second escape couldn't be an accident.

Some time past midnight, Ian hustled past the guards. He was so nervous, it took three attempts to get past the retinal scanner.

It had to be sabotage.

In Lab 5, the tobianos wrestled one another. The overos stared at imaginary mice. Chess's enclosure was empty again.

Before Ian could check to see if his heart was still beating, he heard a demanding meow. He spun.

From between a portable X-ray unit and a file cabinet holding a centrifuge, Chess's alien head poked out. Forgetting every protocol he'd ever written about touching lab subjects, Ian squatted down and picked up the cat. Gently he set Chess back in the enclosure and closed the glass door.

Chess butted the door with his head, rubbed his side against the glass, then lashed and crimped his harlequined tail.

The door clicked open. Ian caught it and pressed it shut. Frowning, he held his palm against it.

The cat walked in front of the door. It unlocked.

"What's happening?"

Ian then realized Chess's tail was at the same height as the retinal scanner. The scanners read blood-vessel patterns. Chess's blood vessels were close to the skin in his tail.

The scanner was reading his tail.

"Like zebra codes at the grocery store." Ian grinned. "It must be a simple default pattern."

He reached over and picked up a roll of masking tape, and ripped off a piece. Opening the door, he blocked Chess's escape with his stomach and covered the inner scanner with the tape. He closed the door again.

"Checkmate." ■

**Nye Joell Hardy belongs to the taxon *California coastalis* var. *geekosti*; has sold a dozen stories; works as a food-safety specialist for a gigantic corporation that experienced some trauma with *E. coli* and spinach; and is actually a 'dog person'.**

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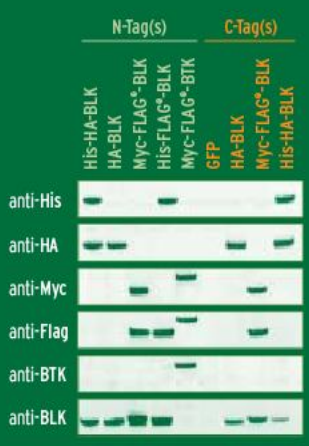


The Western blot analysis of HEK293 cell lysate over-expressing BLK or BTK tagged with indicated epitopes.

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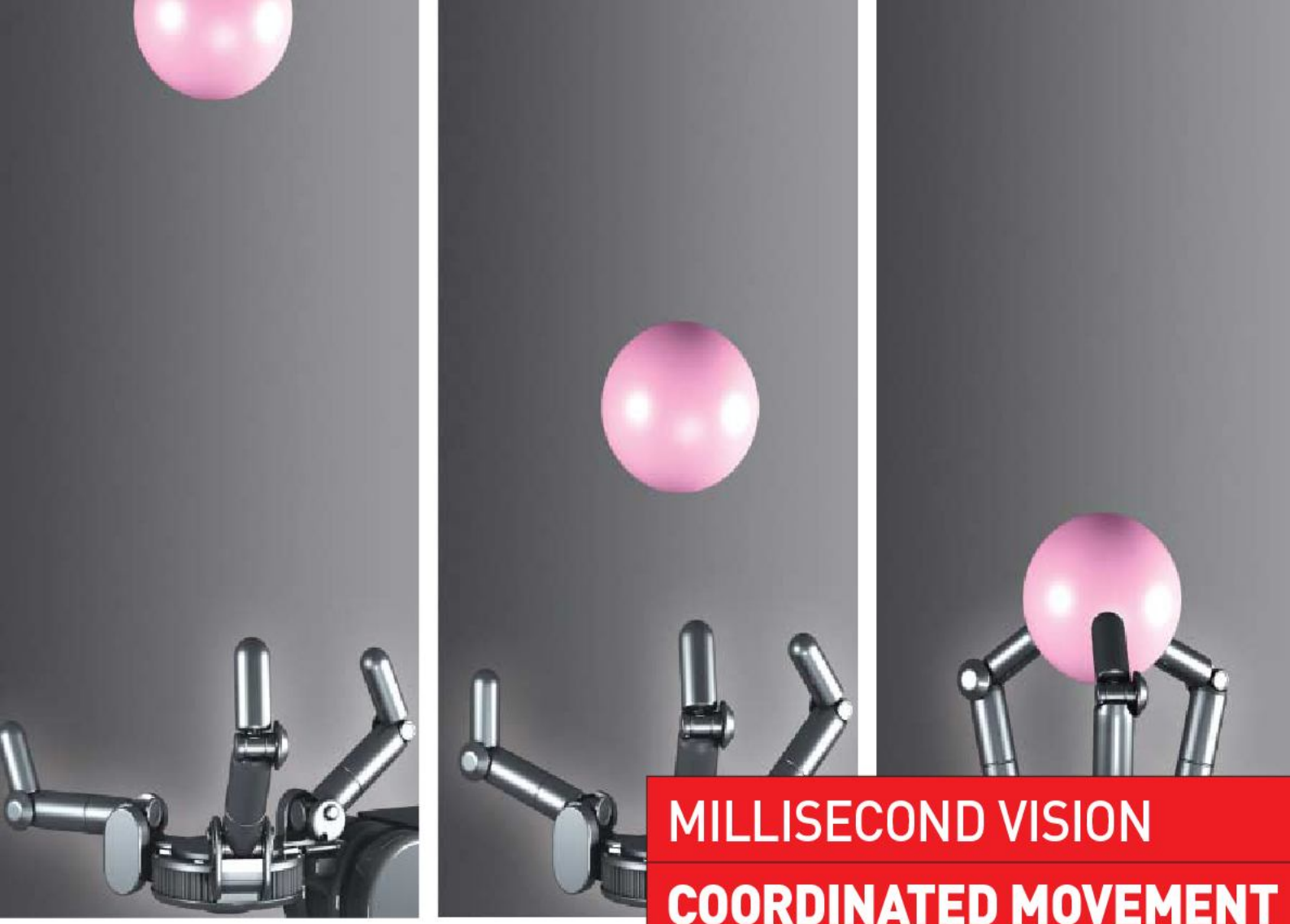

# MILLISECOND VISION
# COORDINATED MOVEMENT

## Hamamatsu Photonics Smart Camera makes high speed and intelligent image processing possible.

It needs to be seen to be truly appreciated. The ball drops. It picks up speed during a free fall. Then three pincers grab it with perfect timing. Even if the ball is off center, the pincers adjust, bouncing the ball from one to the other before securing it. The pincers know what to do.

The heart of this precision robotic system is Hamamatsu Photonics Smart Camera. Conventional cameras used in artificial vision only capture 30 frames per second. Hamamatsu Photonics Smart Camera uses a column parallel vision system that can capture and analyze images, and direct mechanical movement, in less than a millisecond.

The system is based on a parallel structure in which each pixel employs its own dedicated processor. The parallel system is streamlined into a compact controller board.

This system will enable faster and more precise tracking and analysis- a boon for applications in the automation of factory equipment, vehicle and robot control, and inspection, as well as microscopy, the tracking of eye movements, and the analysis of movement in athletics.

Hamamatsu Photonics Smart Camera is being developed in ongoing cooperation with the University of Tokyo, Ishikawa Namiki Komuro Laboratory.

**Hamamatsu Photonics' expertise with light has revealed an elegant solution to a complex problem.**

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